Preliminary Study on Optimization of Ethanol Production from *Citrus aurantifolia* Fruit Juice Using *Saccharomyces cerevisiae*

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Ethanol is an important chemical product with emerging potential as a biofuel to replace fossil fuels. An ecofriendly bio-ethanol is one of the alternate fuels that can be used in unmodified petrol engines with current fuelling infrastructure and it is easily applicable in present day combustion engine, as mixing with gasoline. The objective of the study is to determine the effect of culture conditions & medium composition to increase the yield. Sugar concentration before & after the fermentation was 11° Brix& 3.6° Brix respectively.The sour orange juice was inoculated with *Saccharomyces cerevisiae* (baker's yeast- 2 g/L) in the fermentation media(100ml, sour orange fruit juice: distilled water = 1:3) composed of 10 g/L yeast extract, 10 g/L KH_2PO_4, 2 g/L $(NH_4)_2SO_4$, 2g/L Peptone and 0.5 g/L $MgSO_4 \cdot 7H_2O$ and allowed for fermentation for 24 hoursat room temperature. The amount of ethanol produced from the orange juice was 0.8 % (V/V) at room temperature after 24 hrs of fermentation. The conditions were optimized sequentially by changing one factor at a time while keeping the other variables constant. When different nitrogen sources such as urea, ammonium sulphate, ammonium carbonate and ammonium nitrate were used in the fermentation media, highest ethanol production (alchocol yield 1.0 %, SD = 0.1225) was obtained in the medium containing urea. When the amount of initial yeast inoculum was optimized as 5 g/L, ethanol yield was increased by 1.10 times (alcohol yield 1.10 %, SD = 0.1000) than the control which contained the amount of initial yeast inoculum 2 g/L. When the fermentation temperature was optimized as 35 °C, ethanol yield increase was increased by 1.09 times (alcohol yield 1.20 %, SD = 0.1789) than the temperature non-optimized (30 °C) condition. When the rotation speed of the media was optimized as 150 rpm, ethanol yield was increased by 1.08 times(alcohol yield 1.30 %, SD = 0.1225) than the control speed of 100 rpm. When basal medium containing urea was inoculated with *S. cerevisiae* (5 g/L) and allowed to ferment at 35 °C at 150 rpm for 24 hrs, the ethanol yield produced was 1.30 % (SD = 0.1414). When different concentration of the citrus juice (5 %, 10 %, 25 %, 50 %, 100 %) was used, the ethanol yield was significantly increased by 3.75 times (alcohol yield 4.50 %, SD = 0.1414) with 100 % of sour orange juice, than the control. When the amount of the

nitrogen source was optimized as 0.1 g/100ml, ethanol yield was increased by 1.06 times (alcohol yield 4.80 %, SD = 0.1414) than the control of 0.2 g/100ml. When different carbon sources such as glucose, maltose, sucrose & dextrose were used in the fermentation media, highest ethanol production (alcohol yield 5.60 %, SD = 0.1000) was obtained in the medium containing sucrose. When the amount of the carbon source was optimized as 10 g/100ml, ethanol yield was significantly increased (alcohol yield 10.6 %, SD = 0.146) than the control of 2 g/100ml. When the pH of the medium was changed from 3.0 – 8.0, significantly higher ethanol yield (11.50 %, SD = 0.100) was obtained when pH of the media was kept at 4.0, than the control pH 7.0. Since there was no significant change in the alcohol yield observed between the different incubation periods of the media (24 hrs, 48 hrs, 72 hrs & 96 hrs), it was decided to use 24 hrs as the incubation period for the future experiments. After the optimization of all these culture conditions & medium composition, the bioethanol yield was significantly increased (14.4 times, 11.5 %) than the non-optimized conditions. Large scale fermentation study should be carried out with bioreactor to determine whether this finding could be commercialized.

Key words: Baker's yeast, Bioethanol, Fermentation, Sour citrus fruit, Urea