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Optimization Studies for Bioconversion of Corn Steep Liquor to Ethanol by *Saccharomyces cerevisiae* Strain Ka-1 and Aan-2

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ostract

o strains KA-1 & AAN-2 of *Saccharomyces cerevisiae* were used as a tool for fermentation and the studies for imization of different parameters for ethanol yield from corn steep liquor were undertaken. The maximum yield was ained after 72 hr. at 30 C, 120 rpm in shake flask fermentation. Ethanol production by shake flask fermentation revealed t strain AAN-2 of *S. cerevisiae* had better results as compared to KA-1 which would be a good candidate of ethanol nentation on industrial scale.

roduction

anol is colorless, solvent, volatile in nature having ning taste and spritious smell. Because of its high latent t of vaporization and low air fuel ratio as compared to oline, it can be used as higher inducted fuel energy sities than gasoline (Sherman & Kavasmaneck, 1978). e last three decades, Brazilian proalcohol program and an National Research Council elaborated that bioenergy e form of methane and ethanol produced by fermenting ohydrates present in agro-industrial and animal wastes neet the requirement in gaseous and liquid fuels. The sts are the cheapest source and widely used in alcoholic nentation because of their high growth rate and high otolerance activity during ethanol fermentation.

terials & Methods

ure preparation: A known *Saccharomyces cerevisiae* in KA-1 was provided by microbiology research ratory, Quaid-i-Azam University, (MRL-QAU) Islamabad the other commercially purchased strain AAN-2 were drated in malt medium (Thom and Church, 1926) for e days. The same cultures were re-inoculated twice 24 hrs. in modified malt medium containing 17 g/L of extract, peptone 5 g/L with pH 4.5 at 35°C for 24 hr. aker incubator. Both cultures were then transferred to yeast extract peptone glucose (MYPG) medium aining 3 g/L agar-agar (Choudhary *et al.*, 1986) and the oculations were done for one week. Microbial hology was checked by wet preparations.

fication of corn steep liquor: Corn steep liquor, provided RL-QAU, Islamabad was clarified by diluting it with 1.5 s of distilled water, pH and temperature was tained by the method of Choudhary *et al.* (1986) in ng water bath at 80°C for 5 minutes and then allowed ttle the solid material over night at room temperature. supernatant was filtered and used in fermentation es. Standard curve of ethanol absolute (Merck) was mined by preparing serial dilutions and estimated by oxidation method at A_{600}. Sugar concentration (Miller, 1959), moisture & ashes (Micard, V., 1996), total viable count (Sherply, 1960), growth rate, total dry cell (Mahmoud *et al.*, 1977) were also determined accordingly.

Optimization: The optimization parameters for maximum ethanol production were undertaken by shake flask fermentation such as; influence of different volumes (10-100 ml. v/v) of non clarified & clarified corn steep liquor, age of inoculum (6-96 hr.), size of inoculum (10-50% v/v), different chemicals (media% w/v), incubation period (6-96 hr.), agitation (100-150 rpm), pH (3-8.5), temperature (26-40°C) on ethanol production.

Estimation: After every optimization parameter, the fermented corn steep liquor was subjected to fractional distillation column for over night at 76°C and distillates were later examined by oxidation method of Choudhary *et al.* (1986).

Results

Ethanol production was estimated in different volumes of clarified and non clarified corn steep liquor and found that 100 ml of the substrate is suitable for high ethanol yield. The results spectrophotometer (Shimadzu, Japan) at 600 n.m. showed that strain KA-1 & AAN-2 produced 0.23 and 0.25 and 0.25 and 0.28 per cent ethanol from non clarified & clarified corn steep liquor (Fig. 1 & 2). Age of inoculum 72 hrs. and 20 per cent size of inoculum (v/v) resulted 1.18 & 1.59 and 2.38 & 3.19% ethanol production by strain KA-1 and AAN-2 respectively (Fig. 3 & 4). Different chemicals at different concentration were supplemented to corn steep liquor and results showed that KA-1 & AAN-2 gave high yield of ethanol 3.76 & 4.26 on media-I & III respectively (Fig. 5). Results of maximum ethanol production from corn steep liquor at different incubation periods, agitations, pHs and temperatures revealed that strain KA-1 & AAN-2 produced 3.76 & 4.27 after 72 hr., 3.78 & 4.28 at 120 rpm., 3.78 & 4.29 at 4.5 pH and 4.22 & 4.56 at 30°C temperature. (Fig. 6,7,8 & 9).

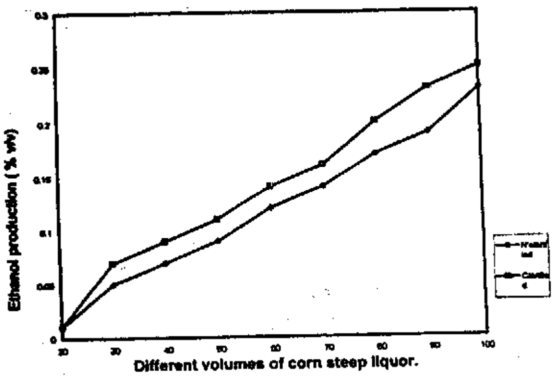

Fig. 1: Influence of non clarified and clarified corn steep liquor at different concentrtion (ml) on ethanol production by S. Cerevisiae strain KA-1 after 05 per cent inoculum, 110 rpm, pH 4.5, 72 hr.

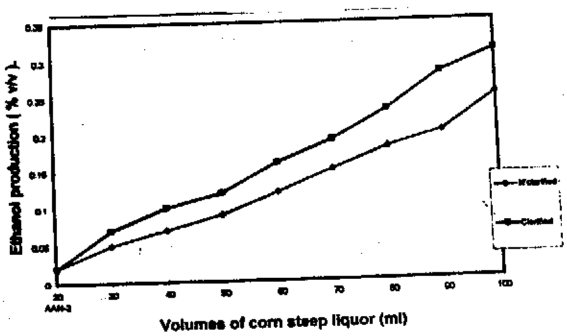

Fig. 2: Influence of non clarified and clarified corn steep liquor at different concentrations (ml) on ethanol production by S. Cerevisiae strain AAN-2 after 5 per cent inoculum, 110 rpm, pH 4.5 after 72 hr.

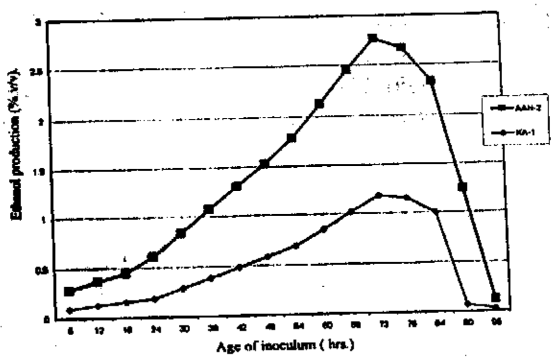

Fig. 3: Influence of age of inoculum (hr.) On ethanol production from corn steep liquor by S. Cerevisiae strain KA-1 and AAN-2 at 32°C, 110 rpm, pH 4.5.

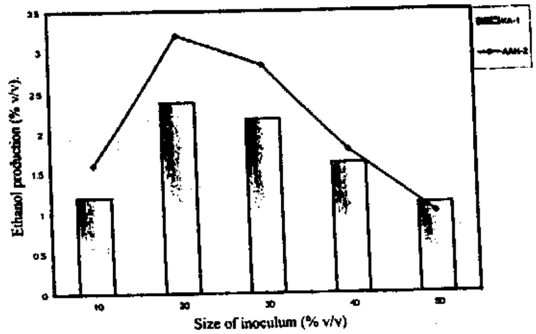

Fig. 4: Influence of size of inoculum (% v/v) on ethanol production from corn steep liquor by S. Cerevisiae strain KA-1 and AAN-2 after 72 hr., 32°C, 110 rpm, pH 4.5.

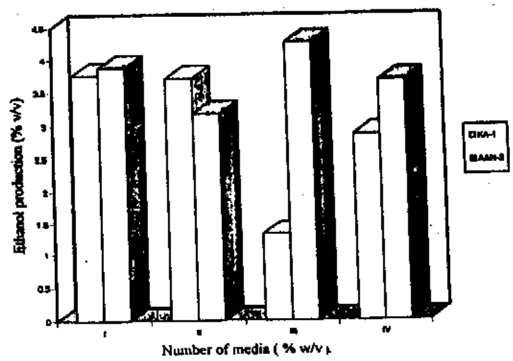

Fig. 5: Influence of different chemicals (media) supplemented to corn steep liquor on ethanol production by S. Cerevisiae strain KA-1 and AAN-2 after 72 hr., 32°C, 110 rpm, 20 inoculum pH 4.5.

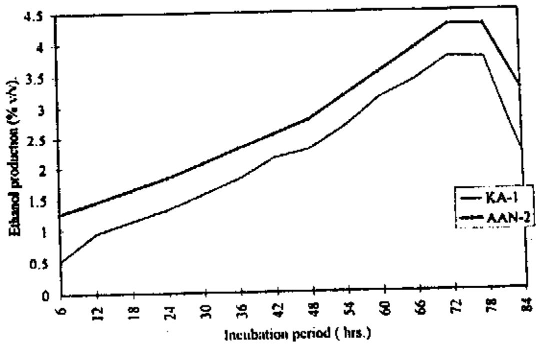

Fig. 6: Influence of incubation period (hrs.) On ethanol production from corn steep liquor by S. Cerevisiae strain KA-1 and AAN-2 with media I and III at 32°C, 20 per cent inoculum size, 110 rpm, pH 4.5.

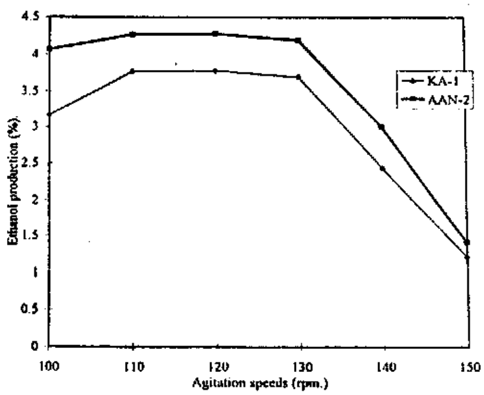

Fig. 7: Influence of agitation speed (rpm) on ethanol production from corn steep liquor by S. Cerevisiae strain KA-1 and AAN-2 at 32°C, 20 per cent inoculum size, pH 4.5 after 72 hrs. Post incubation.

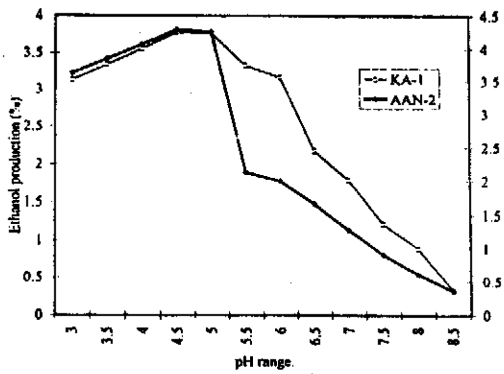

Fig. 8: Influence of pH on ethanol production from corn steep liquor by S. Cerevisiae strain KA-1 and AAN-2 at 32°C, 20 per cent inoculum size, 120 rpm after 72 hrs. post incubation.

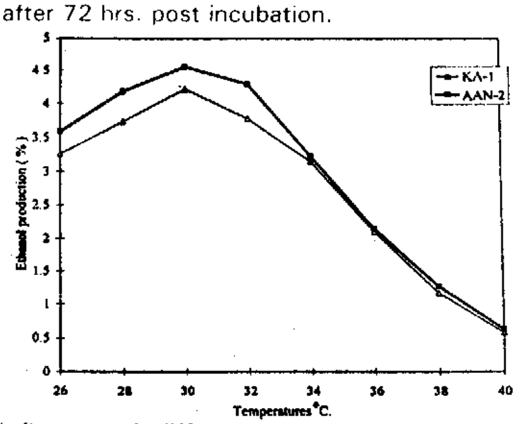

Fig. 9: Influence of different temperatures on ethanol production from corn steep liquor by S. Cerevisiae strain KA-1 and AAN-2 at 20 per cent inoculum size, 120 rpm, pH 4.5 after 72 hrs. post incubation.

Discussion

During the recent years, ethanol production from sugar crops play a splendid role to overcome the global colossal oil crises of the past 30 years. Present study deals with ethanol production from starchy materials (corn steep liquor) having 50-65% starch by *Saccharomyces cerevisiae* which proved to be the spectacular sugar & microbial sources respectively. Fermentation is one of the oldest chemical process known to man is widely used to make variety of useful products by enzymatic hydrolysis of sugars e.g. starch by the action of exoenzymes e.g. amylases, maltase.

Different parameters were used for ethanol production from corn steep liquor by shake flask fermentation. Sugar crops are considered to be highly favourable crops for ethanol production having high content of fermentable sugars. Dried cultures were rehydrated in different media for enhanced yeast growth. Different volumes of non clarified and clarified corn steep liquor were used for maximum ethanol production because it may contain many substances besides sugar viz. mud & ashes which would have the effects on the final yield of ethanol. During shake flask fermentation, little oxygen is required to stimulate the enzymatic activity of the yeast during fermentation process and also maintains the cellular viability of the fermenting yeast.

Age & size of inoculum can vary according to the type of micro-organisms and their species. It is due to the fact that slight increase in viable count resulted in high fermentation rate decreasing total time and increasing the ethanol production. Addition of different chemicals (media) to corn steep liquor could result in the enhanced growth and have the stimulatory effects on ethanol production during fermentation process. Various agitation speeds were undertaken which is the source of homogenicity of chemicals and the oxygen available in the flasks for micro-organisms. Ethanol fermentation was undertaken at different pHs and pH below 3.0 and above 5.5 resulted in drastic reduction and inactivation of cellular growth of yeast.

Temperature is a significant tool in ethanol fermentation process. Temp. 30°C was proved to be an optimum temperature for yeast multiplication. Elevated temperatures lead to slower cell build up and its inactivation plus reduced yield of ethanol. Incubation period along with temperature have the increased efficiency in fermentation. It is generally believed that higher temperature and reduced incubation period could initiate a low cell mass because of the accumulation of ethanol at higher temperature. On the other hand lower temperatures could initiate high yield of ethanol after prolonged incubation.

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