Optimization Studies for Bioconversion of Corn Steep Liquor to Ethanol by
Saccharomyces cerevisiae Strain KA-1 and AAN-2

Agha Asad Noor and Abdul Hameed*
Shah Abdul Latif University, Khairpur, Sindh,
*Quaid-i-Azam University, Islamabad, Pakistan

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

Introduction
Ethanol is colorless, solvent, volatile in nature having
pleasant taste and spurious smell. Because of its high latent
temperature and low air fuel ratio as compared to
gasoline, it can be used as an alternative fuel energy
resource. Over the last three decades, Brazilian pre-alcohol program and
the National Renewable Council elaborated that bioenergy
is a form of methane and ethanol produced by fermenting
agro-industrial and animal wastes to meet the requirement in gaseous and liquid fuels. The
result is the cheapest source and widely used in alcoholic fermentation because of their high growth rate and high
resistance to temperature during ethanol fermentation.

Materials & Methods
Preparation: A known Saccharomyces cerevisiae
strain KA-1 was provided by microbiology research
department, Quaid-i-Azam University, (MRL-QAU) Islamabad
the other commercially purchased strain AAN-2 were
diluted in malt medium (Thom and Church, 1926) for
three days. The same cultures were re-inoculated twice
at 24 hrs. in modified malt medium containing 17 g/L of
yeast extract peptone 5 g/L with pH 4.5 at 35 °C for 24 hr.
in a shaker incubator. Both cultures were then transferred to
3 g/L agar medium (Choudhary et al., 1986) and the
incubations were done for one week. Microbial
phology was checked by wet preparations.

Utilization of corn steep liquor: Corn steep liquor, provided by
RL-CWA, Islamabad was clarified by diluting it with 1.5
water bath at 80 °C for 5 minutes and then allowed
temperature. The supernatant was filtered and used in fermentation
batches. Standard curve of ethanol absolute (Merck) was
extracted by preparing serial dilutions and estimated by

Optimization: The optimization parameters for maximum
ethanol production were undertaken by shake flask
ermentation 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 (Shimazu, Japan) at 600
nm. 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 and 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, agitation, 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 concentrations (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 per cent 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.
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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