ISSN: 0974-2115
www.jchps.com Journal of Chemical and Pharmaceutical Sciences

Comparative study on the effect of additives using *Avena sativa* (Oats) and *Cicer arietinum*(Bengal Gram) for ethanol production

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ABSTRACT

Additives like *Avena sativa* (Oats) and *Cicer arietinum* (Bengal Gram) were used as supplements to evaluate their effects in alcohol production from cane molasses using *S.cerevisae*. Various concentrations of these additives (1-5%) were used to enhance the rate of ethanol production in batch fermentation. *Avena sativa* increases ethanol production (68.2 w/v) as compared to control experiments (64.1w/v) lacking supplements after 72 hr fermentation. *Avena sativa* showed higher productivity of alcohol about 68.2g/l compared to *Cicer arietinum* which produced a yield of 67.4 g/l. In general, addition of *Avena sativa* and *Cicer arietinum* supplements to the fermentation medium showed more than 40% improvements in ethanol production using cane molasses sugar concentration.

KEY WORDS: Biofuel, Yeast, Fermentation, *Avena sativa* (Oats), *Cicer arietinus* (Bengal Gram).

1. INTRODUCTION

Fermentation process of clean fuel production has considered as a most important and yeast strain based on its application of field production by improving substrate utilization range and several manipulations has been carried without any success (Mobini, 2007; Sreenath, 1996). The various substrates of cashew, yam bean and certain pulses has been tested for strain development and production yield improvement of which cashew has been expensive with less production yield. The optimum conditions for the production of ethyl alcohol have not been structured for improved results and it has to be optimized (Yu, 2007; Rocha, 2007; Campos, 2002). The vegetable waste materials have been taken into consideration for production of biofuel with optimized condition on their non-edible portion which carries organic solids (Kulkarni, 2001).

Fermentation process of ethanol production has been used by microbial culture throughout the world and Yeast (*Saccharomyces cervisiae*) has been traditionally major microbial load to metabolize sugars into solution of ethanol and carbon dioxide (Pursell, 2007). Ethanol production from renewable solid sources helps to reduce greenhouse gas emissions and ethanol will poses high octane number by decreasing the vapor pressure with clean burning characteristics of no net carbon dioxide and sulfur monoxide emissions (Lin and Tanaka, 2006). The lingo cellulosic materials in the form of corn, sorghum, wheat, rice and sugarcane or sugar beet and a byproduct of sugar industry molasses has been extensively used to produce ethanol and these natural sources contains rich protein and total sugar content helps the yeast or bacterial culture for an ideal substrate for metabolizing sugars into fermentable sugars for production of high Octane ethanol (Kheshgi, 2000; Dubois, 1956; Szambelan, 2004; Shigechi, 2004). With inevitable acceptance of fermented production of biomass based biofuels has sustainable development with resource available to convert into secondary energy in more amount has bend for more plantations which contributes to social aspect of sustainability (Vanden, 2000; Monique, 2003; Lynd, 1996; Soccol, 2010; Sun and Cheng, 2002; Tomas, 2009).

In this current research the ethanol production by *Saccharomyces cervisiae* has been carried out with additive like *Avena sativa* and *Cicer arietinus* and the effective parameters has been verified for the preparation of fermentation medium with adequate supply of every ingredients.

2. MATERIALS AND METHODS

2.1. Collection of the Molasses: The molasses samples were collected in Tiruttani sugar mill Ltd, Tamilnadu, South India and samples were collected in large sterilized bottles and brought to the laboratory. The molasses samples were filtered through cotton to remove suspended solids.

2.2. Preparation of media for fermentation: The various parameters were taken into account on optimization of media for production of ethanol. The parameters were temperature ranging from 24 -50^{0}C, pH ranging from 4-7, concentration of biomass ranges between 0.2- 2.0 g/L with various substrates of sucrose, fructose, maltose, glucose, lactose and additives.

2.3. Fermentation and Its Kinetics: The fermentation process flasks were prepared based on optimization criteria and processed materials were analyzed on following methods, The ethanol content of the fermented broth was determined by measuring specific gravity of the distillate according to the procedure described by Amerine and Ough (1984), and Balasubramaniyan (2008) and cell concentrations in systems were calculated with corresponding dry weight from a standard curve of absorbance versus dry weight (Behera, 2010).

2.4. Statistical analysis: The data of ethanol production using strains was analyzed using one way ANOVA. Where significant difference in ANOVA (p <0.05) was detected by the Fisher's Least Significance Difference (LSD) multiple comparison test which was applied to compare the factor level difference.
2.5. Analysis of product by GCMS: The Thermo GC-Trace Ultra Ver-5.0 was used to analyse the product. The column used was ZB-MS Capillary standard non polar column. All components can be quantitatively analyzed in less than 10 minutes.

3. RESULTS AND DISCUSSION
3.1. Ethanol production from molasses at different concentration at different time duration: The different concentration at different duration of the processing conditions has produced varying ranges of ethanol at 40-61.8 g/l with best showing in 62.1 g/l in concentration of 1.4g/l of substrate in 120hrs of fermentation process and the processed conditions have been tabulated with calculated vales of ethanol production in table 1.
3.2. Ethanol production from molasses at different Temperature: The different time duration on ethanol produced were analyzed for its fermentation constants and determined the presence of factors at different proportionate in table 2.
3.3. Ethanol production from molasses at different pH: The varying ph and production of ethanol as shown conversion rate of 50-58 g/l with specific best observed in pH of 5.5 with production yield of 0.320 on conversion limit showing 57 g/l is tabled in table 3.
3.4. Ethanol production from molasses at different Substrate: On different substrate of glucose, sucrose, fructose, maltose and lactose the cell yield has been higher in maltose with product productivity of 0.5 at conversion of 62 g/l of substrate used which is tabulated in table 4.
3.5. Ethanol production from molasses using different additives: *Avena sativa* increases ethanol production (68.2 w/v) as compared to control experiments (64.1w/v) lacking supplements after 72 hr fermentation. *Avena sativa* showed higher productivity of alcohol about 68.2g/l compared to *Cicer arietinum* which produced a yield of 67.4 g/l.
DISCUSSION
Industrial ethanol production of ethanol from various starchy components from corn, wheat, potato and cassava root has been reported and grounded for production of ethanol at higher limit (Lindeman and Roccinnicli, 1979; Kadam and McMillan, 2003; Wilke, 1981; Sasson, 1990). Considering the lingo cellulosic plant which has more complex substrate than starch which carries mixture of polymers likes of cellulose, hemicelluloses and lignin. The delignification process to liberate it from carbohydrate which is to produce free sugars and fermented for ethanol production has been researched and carried over for decades has been cited (Yu and Zhang, 2004; Tampier, 2004). The microbial flora of anaerobic thermophilic bacteria of *Clostridium thermocellum* (Ingram, 1987), *Monilia sp.* (Saddler and Chan, 1982), *Neurospora crassa* (Gong, 1981), *Aspergillus sp* (Sugawara, 1994), *Trichoderma viride* (Ito, 1990) and *E.coli* strains (Millichip and Doelle, 1989) for cellulose to ethanol biotransformation. The multi different strains of yeast species has been used to produce ethanol as main fermentation product with highly bio convert able sugars (Vallet, 1996; Todor and Tosonka, 2002; Camacho-Ruiz, 2003; Kiran, 2003; Sanchez, 1999).

Cauliflower waste (*Brassica oleracae*) vegetable waste contributes to 40-60 % of its total weight and consists of 85.5% of organic constituents with 17% of total sugars, 16.6% of cellulosic content and 14.9% as crude protein as its primary constituents with minerals of calcium, magnesium, sodium, potassium and sulfur has yielded significant yield of ethanol against standard starchy substrates (Oberoi, 2007). Diluted molasses with *Saccharomyces cervisiae* MTCC 178 has incorporated by dried cauliflower waste by 15% as increased in ethanol production when comparing to clear 36% production on molasses with yeast efficiency by 49%. The final fragmented cell biomass and ethanol production yielded 70.11% as supplementation to improve it production based on organic nature (Gurpreet singh, 2007).

Neelakandan (2009) has optimized the production of Bioethanol using cashew apple juice using immobilized yeast cells with fermentation parameters concentration of substrate, pH, temperature and inoculum variations as yielded ethanol of 7.62% at pH 6.0, optimum temperature of 32.5°C with inoculum percentage of 8 (v/v) under controlled condition. Flour prepared from *Solanaum tubersum* (Potato) tubers was homogenized and slurry of α-amylase and starch has been used for saccharification with glucoamylase has resulted in total of 15.2% reducing sugars and hydrolysated by fermentation with Saccharomyces cervisiae HAU-1 for 48 hrs resulted in production of ethanol of 56.8% g/l (Rani, 2010).

Yam been used as an substrate additive to Saccharomyces cervisiae TISTR 5339 as batch, fed batch and repeated batch fermentation has showed significant yield of 12.01±0.33 g/l ethanol yield on comparison with immobilized cell s of 0.59±0.02 g ethanol/ g sugar (Waesarat, 2012). Higher fermented yield has been achieved through process of simultaneous saccharification and fermentation of converted lingo cellulosic content and comparing between yeast species of Saccharomyces cervisiae and Kluyveromyces marxinus of its potential in ethanol production showed significant enhancement in later species with 65% activity (Danielo, 2014).

Table.1. Production of ethanol from molasses at different concentration by yeast

Duration (hrs)	0.2 g/l	0.4 g/l	0.6 g/l	0.8 g/l	1 g/l	1.2 g/l	1.4 g/l	1.6 g/l	1.8 g/l	2.0 g/l
24	8	9	11	12	14	15.8	17.2	18	17.4	17.2
48	12	14	16	18	22	29	36.1	38	35	34
72	16	18	20	24	29	37	43.5	45	44.6	44
96	20	25	28	32	36	43	59.8	60	61	60
120	24	30	24	38	42	51	62.1	61.9	61.8	61.7
144	29	36	40	42	48	52	61.2	61	60.8	60.5

Table.2. Production of ethanol from molasses at different Temperature by yeast

Fermentative Analysis	25	30	35	40	45	50	55
Ethanol (g/L)	34.2	35.1	36.4	38.2	40.8	40.2	39.8
Biomass (g/L)	0.89	0.91	0.92	0.96	1.08	1.02	1
Cell yield	0.06	0.065	0.07	0.08	0.09	0.07	0.06
Ethanol yield	0.21	0.24	0.26	0.30	0.320	0.31	0.29
Substrate uptake	0.92	0.95	0.97	0.99	1.07	1.01	0.95
Product productivity	0.31	0.34	0.36	0.38	0.42	0.40	0.34
Conversion rate	42	45	48	52	57	55	51

Table.3. Production of ethanol from molasses at different pH by yeast

Fermentative Analysis	4	4.5	5	5.5	6	6.5	7
Ethanol (g/L)	38.9	39.2	39.4	40.8	39.2	35.2	30.1
Biomass (g/L)	1.04	1.05	1.06	1.08	1.06	0.98	0.81
Cell yield	0.06	0.07	0.07	0.09	0.08	0.05	0.04
Ethanol yield	0.30	0.31	0.31	0.320	0.31	0.25	0.15
Substrate uptake	1.02	1.04	1.05	1.07	1.05	0.98	0.68
Product productivity	0.34	0.36	0.37	0.42	0.38	0.26	0.14
Conversion rate	51	51	53	57	52	48	31

Table.4. Production of ethanol from molasses at different substrate by yeast

Fermentative Analysis	Glucose	Fructose	Maltose	Sucrose	Lactose
Ethanol (g/L)	40.7	40.8	42.8	39.8	41.2
Biomass (g/L)	1.09	1.08	1.1	0.9	0.98
Cell yield	0.08	0.09	0.1	0.09	0.99
Ethanol yield	0.33	0.320	0.4	0.34	0.35
Substrate uptake	1.08	1.07	1.2	0.9	1
Product productivity	0.43	0.42	0.5	0.4	0.4
Conversion rate	56	57	62	52	60

Table.5. GCMS data for Ethanol Production

Additives	Retention Time	NIST Database
Control	1.18	Ethanol
Cicer arietinus	1.33	Ethanol
Avena sativa	1.50	Ethanol

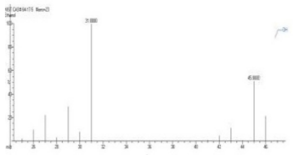

Figure.1.GC MS data for ethanol standard

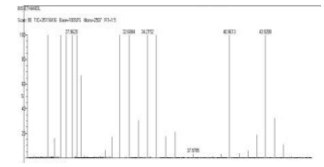

Figure.2.GC MS data for ethanol after addition of additive *Cicer arietinum*

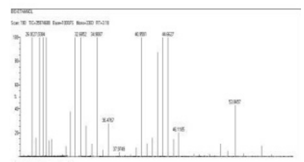

Figure.3.GC MS data for ethanol after addition of additive *Avena sativa*

4. CONCLUSION

The ethanol production from the biomass has yielded more efficiency and percentage when chemically synthesized with lower interests resulted in clear clean biofuel. The substrates and additives has been boost to the development of biomass generated fuel produces more yield co efficiency and production of reliable convertible biomass for further process. The above research work has been carried for the benefit of the ethanol production improvement from the additive and it showed better results when compared to the normal ethanol production.

Conflict of Interest: Authors declare no conflict of interest on research work carried.

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