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Promotion of Alcohol Production using high Gravity Fed-Batch Alcoholic Fermentation through Novel Yeast Strain

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Abstract— Fermentation is well known biological process to produce the alcohol by using raw materials as molasses, grains etc. The performance of molasses based distilleries are day by day improved towards high efficiency, lowest sugar loss and high yield. In many distilleries, earlier and at present the operation mode of fermentation process is "batch fermentation" which gives the less efficiency 80 to 85 % with poor quality alcohol and spent wash generation is about 8 to 15 lit/lit of alcohol produced. Later on it is developed as "continuous fermentation" which gives high efficiency 88 to 90% and with better quality of alcohol and spent wash generation is about 3 to 4 lit/lit of alcohol produced by adopting spent wash recycle to fermenter and treatment of spent wash in integrated evaporation with distillation system. But In India distilleries effluent i.e., spent wash has high organic load which causes Major water pollution so these industries are comes under "Red category" and it is necessary to improve this process and reduce the spent wash generation as much as possible along with high fermentation efficiency and less energy consumption for separation of alcohol in downstream process of distilleries. So this presentation is to introduce the new mode of operation named as "High gravity Fed- batch alcoholic fermentation" of cane molasses. The pilot trial is taken by using the developed yeast stain "*Scizosacharomyces Pombe*-Fed" with upgraded feeding pattern. Process parameters are experimented and studied in "Shree Renuka Sugars Ltd. Havlaga unit" and the results high efficiency of about 89 to 90 % and high yield of alcohol in fermented wash of about 11.8 to 12.5% so as to reduce the spent wash generation is about 2.2 to 2.4 lit/lit of alcohol produced which will helps to achieve one of the strict norm of Pollution Control board "Zero liquid Discharge" with post treatment of Bio-composting and incineration of high brix spent wash(55 to 60 % Solids) in specially designed boiler.

Keywords— Batch fermentation, continuous fermentation, Fed- batch fermentation yeast microorganism, Zero liquid discharge, Red category.

I. INTRODUCTION

In India most of the molasses distilleries are producing alcohol total installed capacity is 4230 million lit per Annam 2016[1]. Fermentation process is the upstream biological process of alcohol production and it is carried out in many mode of operation like batch, cascade continuous, (low gravity media) in which the sugar source is diluted and maintained the moderate sugar concentration in the media nearly about 7 to 8% v/v depends on the mode of operation. (4)The fermented broth containing low Alcohol concentration not only makes the alcohol is extracted by distillation highly energy-intensive, but also generates more effluent generation that needs to be treated by multistage evaporation, costing even more energy. As a result, many attempts have been made to achieve higher Al% concentration at the end of fermentation, High gravity (HG) alcoholic fermentation technologies were proposed in the earlier and applied which made the Al% concentration at the end of fermentation increase up to 10 to 11%.(2,5) But the variations observed that increasing in the residual sugar level at the end of the fermentation, which will increases raw material consumption, & also increase the difficulties of process control if the developments have not been done [2].

Research in yeast physiology has revealed that many strains can tolerate far higher ethanol concentration usually without any conditioning or genetic modifications that risk making the modified strains lose some of their original Characteristics [3]. Therefore, high gravity (HG) Alcoholic fermentation technologies using media containing sugar of about 22% in order to achieve more than 12% (v/v) Al%. As the cost of fuel ethanol production is mainly from raw

material consumption the residual sugar at the end of fermentation is strictly controlled at a level of 1.5 to 2% in industry.

II. RELATED WORK

To evaluate alcohol production using high gravity fed-batch alcoholic fermentation through novel yeast strain.

III. METHODOLOGY

Many microorganisms being exploited for alcohol production, the genus of Saccharomyces cerevisiae, Zymomonus mobilis schizosaccharomyces pombe etc. Currently, either alcoholic beverage or industrial fuel, is being produced by fermentation using the above strains. Acclimatization of the yeast strain was done in laboratory scale at high alcohol concentration 12 to 13% and high osmotic pressure, and high sugar concentration 18-20% the same strain is used for the new mode of operation I.e. improved "High gravity fed-batch alcoholic fermentation" and the stain most intensively developed species because it possesses some "superior characteristics" compared to above strains. Initially parent yeast is used to prepare the culture in lab stage of 50 ml volume of diluted molasses medium of Specific gravity 1.050 with addition of nutrients as urea 40 to 50 ppm as nitrogen source. Acclimatization of the parent yeast is done adopting the yeast culture in 5Lit capacity of small scale culture vessel in laboratory with high gravity medium up to media Sp. Gr 1.090. With this Acclimatized stain of "*Scizosacharomyces Pombe*-Fed" we have done trial in large scale fermenters located at Shree Renuka sugars Ltd at Havlaga unit Karnataka India.

Culture vessel setup:
Set up was for the culture preparation vessels CV-1,CV-2,CV-3 ,CV-4 and CV5 Capacity 50 lit, 450 lit, 4000 lit, and 40000 lit and 40000 lit respectively MOC SS 304 ,sterile aeration is provided by the air blower through HEPA filter and Micro filter. To set up the uniform medium the broth mixture and Central agitator, for cooling the media cooling water through jacket and PHE is provided.

Fermentation system:
Three identical fermenter (Bio-reactor) was established of capacity each 900m3 Diameter- 10.1 mtr and Height-11.25 mtr ,Shape of fermenter is cylindrical with bottom coned, MOC- MS (Mild steel) Epoxy coated throughout the internal part of fermenter and the working volume is considered as 90- 95% to the fermenter sterile aeration is provided through air sparger system by air blower of capacity 400Nm3/hr. and the mixing of the raw contents was done by circulating the media with pumping system at a rate of 500 m3/ hr. and to maintain the temperature of wash at 32°c Plate heat exchanger provided to each fermenter with RTD sensor.

Culture Preparation and pitching:
The above acclimatized mother culture is used for the preparation of required quantity of inoculum at every stage of Culture preparation molasses media is sterilized up to 121°C for 15 min and cooled at up to 32°C and retention time of 12 to 16 hours the culture is transfer to next stage as inoculum and finally at the stage of CV-4 yeast Cell count of mother culture is maintained about 550 to 600 million cell /ml with 98 to 100 % vitality, Sp.gr of culture media is 1.024 to 1.028 yeast culture quantity 40 m3 is ready as mother culture for fermentation system.

Buildup of fermenter with Developed seed Culture:
In the fermentation process, the rate of fermentation is depends on the quality of raw material used normally "A" grade molasses is preferred of quality used in this trail is TRS 50 to 51% and UFS- 4.3 %, FS – 46 to 46.5%, During the fermentation in glycolysis pathway under unfavorable conditions and parameters byproducts formation is observed like acetone, aldehyde, methane, higher alcohols namely iso-propyle alcohol amyl alcohol, iso-amyl alcohol etc. So it is necessary to maintain all parameters like optimum temperature of fermenter should be 32°C to 33°C, pH range is 4.4 to 4.6 and is maintained with the dose of Sulfuric acid which will fulfill the additional benefit sulfur content as nutrient. Cell count of the fermenter is should be in the range of 450 to 500 million cells pr ml of sample. Sugar concentration is maintained not more than 12 to 14% so as to prevent osmotic stress on yeast cells. Sterilized air is to be provided by using HEPA and micro filter. Urea and DAP nutrient dosing is given about 40 to 50 ppm for each batch as a nitrogen source by taking the precautions to avoid the contamination in culture preparation.

Feeding and input methods of raw materials:

- Initially the fermenter is charged by the molasses and water media of sugar concentration maintained 7 to 8% and set up gravity 1.055 to 1.060 and level taken of about 30% of the fermenter working volume includes the simultaneous addition of seed culture 75 m3 at the feed rate 30 m3/ hour (9 to 10 % of total volume).
- RT of 3 hour gap is given to the fermenter to activate and improve the cell count of the culture in the low gravity medium.
- After this RT feed gravity increased to 1.140 by adjusting the molasses and water feed up to 4 hours in between water feeding is stopped when Pre-calculated water quantity reaches.
- After 4 hours of above feeding Increase the feed quantity of molasses by 15 to 20% by analysis so that the Sp. Gr of fermenter should not be above 1.090 and

complete the molasses feeding same as per required alcohol % i.e. 12 to 12.8% w.r.to TRS of molasses.

- Aeration is stopped after completion of feeding i.e., up to 18 to 20 hours from the starting feed time.
- The total feeding pattern is completed up to 18 to 20 hour by considering this feeding time to complete the fermentation of one fermenter RT is required 48 to 52 hours.
- The total time taken to complete one batch including emptying of fermenter is 52 to 54 hours.

IV. RESULTS AND DISCUSSION

During the trial of Fed- batch alcoholic fermentation of cane molasses below results are recorded with feed pattern.
Process oscillations observed:
By the observation of initial batches we have given the molasses at feed alcohol 12.0% but due to the less cell count it is observed that the recovery is less. Then the culture feed is raised up to 70-75 m3 per fermenter i.e. 9-10% of total volume then the result was observed that cell count maintained 440 million cells per ml sample which has given the better result. Initially During completion of total feed the Sp. Gr of the fermenter is maintained 1.090 to 1.098 which causes the osmotic pressure due to high sugar concentration the cell growth rate is restricted so the rate of fermentation is reduced so the precaution was taken that sugar concentration in the feed is optimized and specific gravity is maintained not more than 1.084 then the better results are observed in the next batches. Due to less cell count the residual sugar range after completion of fermentation is raised to 2.5 to 2.9% at the initial batches so the culture quantity is raised up to 10% the range of residual sugar came down to 1.9 to 2.0% by this the alcohol recovery is increased.

Positive approaches and achieved results: Retention Time (HRT): The time taken to complete conversion of sugars to alcohol in the above mode of operation is 48 to 52 hours. In which the 18 to 20 hours are required to charge the fermenter. This time was the reduced by modified feed pattern keeping the all observations like cell count, sugar concentration alcohol yield and byproduct formation. These results are similar with the previous studies conducted [2].
Fermentation Efficiency: Initially we have got the fermentation efficiency 83 to 85% because of sugar loss and culture pitching quantity was less because of this the desired Al% was not achieved to minimize the loss of sugar culture pitching quantity is increased and aeration time was increased up to 20 to 22 hours.

Recovery and yield: As per the theoretical calculation by analyzing the sugar content in the cane molasses the recovery of alcohol matches, in practical as per above parameters it is observed that there is no recovery loss in this mode of operation.

Residual Sugars: After the Retention time of 52 to 54 hours the residual content in the fermented broth is about 1.8 to 1.9 % which indicates the negligible sugar (un-fermentable sugars) loss in this mode of operation.

Less energy consumption: At high alcohol concentration (12 -13%) fermented wash required to achieve the installed plant capacity is less so the heat energy required in downstream process to extract the alcohol is less.

Chemical consumption: During the fermentation to control the foaming anti foam agent required is minimized up to 40%.

Less Effluent Generation and its parameters: Spent wash generation in the continuous mode of operation is 3.5 to 4 lit/lit (40 to 45 % solids) of alcohol produced even by adopting the evaporation treatment and without evaporation it is 7.5 to 8 lit/ lit (16 to 18 % solids) of alcohol by counting the 20% recycle of spent wash to fermentation. By the "High gravity fed-batch alcoholic fermentation" mode of operation basically the alcohol produced is 12 to 12.8% so the fermented wash required to reach the plant capacity is 30% less as compared with continuous mode of operation. So initially the effluent produced after distillation the generation of effluent is 30% less so we have achieved the 2.2 to 2.4 lit/ lit (55 to 60 % solids) of alcohol produced with adopting the integrated evaporation and forced circulation evaporation system and without evaporation 5.7 to 5.8 lit/ lit of alcohol produced(20 to 22 % solids)by taking an example of 120 KLPD distillery can treat its spent wash is about 1.5 lit/lit less in this mode of operation so the cost for treatment of effluent treatment is reduced up to 30%.

Feeding pattern and observation of process parameters & its descriptions.

As per the pilot trails the result and feed pattern details given below.
Initially during the first 3 hours feeding it is observed that the growth rate of the yeast cells are enhanced by feeding the molasses and water media having the sugar concentration 10 to 12 % with dosing of nutrients of nitrogen source with sterile aeration. In the next step of feeding 3 hours retention is given so as to achieve the desired cell count about 420 to 450 million cells/ml.

After RT the molasses feed and water feed is given at set up Sp. Gr 1.140 up to 4hours so that the growth rate is increased by aerobic respiration simultaneously an aerobic fermentation also done because of high set up gravity feed

[4]. During this period the analysis shows that specific gravity and residual sugars are increased up to 1.081 and 5.9% respectively.

In the next step of feeding as per analyzed parameters, molasses and water quantity was adjusted so that the optimum osmotic pressure is maintained because if it is high then the yeast activity is reduced and rate of fermentation is affected [5]. After 22 hours aeration is stopped it Leeds to perform the anaerobic alcoholic fermentation to get the desired Al% and it is observed that 12 to 12.5% alcohol is achieved by this mode of operation.

V. CONCLUSION AND FUTURE SCOPE

This is the developed process regarding High gravity fed-batch alcoholic fermentation, based on the results achieved by the use of developed stain "*Scizosacharomyces Pombe*-Fed" and upgraded process feeding pattern, it is concluded that we can run the large scale distilleries more than 200 KLPD by minimizing the heat energy requirement ,less cost of equipment maintenance, no recovery loss and can achieve easily "Zero liquid discharge" by post treatment like Bio-camposting and incineration of effluent in boiler.

Table 1. Trial results and feed pattern of fed-batch fermentation.

Detailed analysis Report of fed-batch fermentation.										
No of Batches	**Molasses used in MT**	**Water used in m3**	**Culture pitching in m3**	**Sp. Gr after molasses feeding completion**	**Final gravity**	**Alcohol content %**	**Residual sugars**	**Observed recovery lit/MT Molasses**	**Fermentation Efficiency**	**Retention time**
1	392	478	65	1.091	1.061	11.6	2.4	248	83.37	52
2	384	480	67	1.088	1.059	11.7	2.2	254	85.31	50
3	384	480	65	1.084	1.056	11.7	2.1	254	85.36	48
4	384	480	65	1.082	1.054	11.6	2.3	251	84.11	50
5	370	480	64	1.080	1.058	11.5	2.1	256	86.01	50
6	370	475	62	1.075	1.060	11.5	2.2	252	84.72	54
7	370	477	65	1.100	1.066	11.3	2.17	250	83.77	54
8	383	482	70	1.075	1.056	12.05	1.98	262	87.81	52
9	368	481	70	1.078	1.057	11.8	2.01	266	89.27	52
10	374	479	70	1.079	1.054	11.9	1.96	263	88.41	50
14	377	482	70	1.078	1.052	12.00	1.90	267	89.46	52
15	392	479	65	1.095	1.060	12.28	2.9	263	88.37	50
16	377	480	70	1.080	1.050	12.50	1.94	279	89.68	48
17	383	481	70	1.078	1.053	12.80	1.86	281	90.55	50
18	386	484	71	1.080	1.053	12.60	1.98	277	89.22	50
19	386	480	70	1.079	1.055	12.80	1.90	280	89.98	52
20	383	486	72	1.079	1.058	12.40	1.96	274	88.15	50
21	380	480	73	1.078	1.052	12.70	1.92	282	90.64	50
22	381	480	70	1.080	1.053	12.72	1.80	281	90.32	48
23	386	480	72	1.085	1.054	12.70	1.86	276	88.95	50
24	386	480	75	1.078	1.053	12.80	1.96	280	89.98	48
25	383	480	73	1.078	1.054	12.80	1.98	281	90.44	48
26	378	480	75	1.080	1.056	12.70	1.89	283	90.98	50
27	381	480	77	1.082	1.054	12.80	1.94	284	91.23	48

Table No 2. Raw material feeding patterns and analysis results of fed batch fermentation

Feed pattern of 900 m3 capacity fermenter and its results								
Time in hrs.	**Culture Qty.**	**Molasses feed**	**Water feed**	**Specific gravity**	**Residual sugars %**	**Cell count million/ml**	**Viable cells %**	**Al %**
Up to 3 hrs.	75m3 @ 25 m3 / hrs.	52 Ton @ 17.5 Ton / hrs.	150 m3 @ 50 m3 / hrs.	1.066	NE	290	90	NE
3 to 6 hrs.	3 hour RT is given with aeration continuous at rate 400 Nm3/ hrs.			1.051	4.1	390	85	NE
6 to 10 hrs.	--	82 Ton @ 20.5 Ton / hrs.	120m3 @ 30 m3/ hrs.	1.065	5.2	410	85	6.4
10 to 20 hrs.	--	116 ton @23.5 Ton / hrs.	210M3 @ 30m3/ hrs.	1.081	5.9	430	82	7.1
	Aeration is stopped to boost the anaerobic alcoholic fermentation							
After 24 hrs.	Under Retention			1.076	4.4	430	80	8.9
After 32 hrs.	Under Retention			1.069	4.01	380	75	9.4
After 40 hrs.	Under Retention			1.061	3.5	360	73	10.8
After 48 hrs.	Under Retention			1.057	3.1	330	70	11.8
After 52 hrs.	Under Retention			1.052	1.9	240	70	12.6

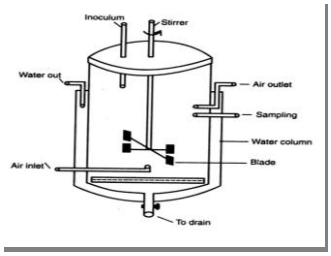

Figure 1. Schematic diagram of fermenter.

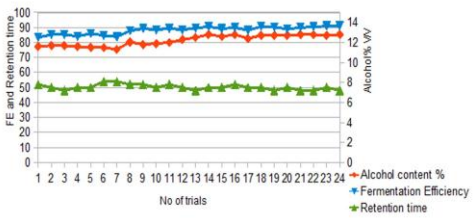

Figure 2. Graphical representation of no of trial in fed-batch alcoholic fermentation.

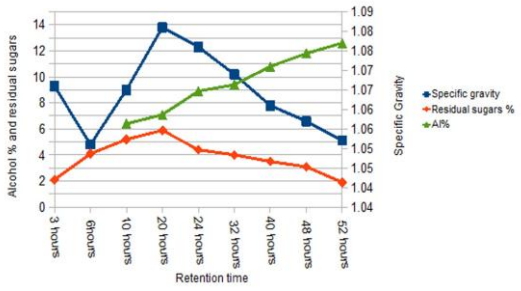

Figure 3. Graphical presentation Variation of Parameters during the fed batch alcoholic fermentation.

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