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POLLUTION MINIMIZATION BY USING GAIN BASED FERMENTATION PROCESS

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ABSTRACT

Distillery sector has been identified as one of the highly water polluting category and grouped under "Red" category and amongst the 17 categories of highly polluting industries. It involved the fermentation of sugars (sucrose) by yeast *S. cerevisiae* and distillation of fermented broth. Molasses is being used as main raw material for carbon source, which contains about 40-50% sugars. Molasses also contains caramelize sugars i.e. Caramel which are non-fermentable and imports color in final effluent. Recently, grains are also being used as raw material in place of molasses, which contains about 65-70% starch. Starch is converted to fermentable sugars i.e. glucose, sucrose etc. by liquefaction and saccharification process using α-amylase and amyloglucosidase enzymes respectively. This results high sugar content in raw material and further saccharification and fermentation take place simultaneously. These enables to achieve 12-14% alcohol in fermented broth as compare to 8-10% alcohol in case of molasses based distilleries. This results in low volume of effluent generation with lesser organic loads (low BOD/COD values) and absence of Caramel in the effluent.

Key words: Distillery, Alcohol, Fermentation, Molasses, Grain**.**

INTRODUCTION

Ethanol (Alcohol) is produced industrially mostly by fermentation of sugars (sucrose) and distillation of fermented broth. Yeast, *Saccharomyces cerevisiae*, is used for fermentation of ethanol from sugar. Numerous models have been proposed to describe the behavior of *S. cerevisiae* under different growth conditions[1,2]. Fermentation may be batch, fed batch or continuous type. There are about 400 distilleries in India, out of which most of them utilize molasses as main raw material. Molasses contains about 40-60% fermentable sugars (sucrose, glucose and fructose) and about 3-10% non-fermentable sugars[3]. Molasses

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also contains caramelize sugars i.e. Caramel, which are non-fermentable and imports color in final effluent. After fermentation and distillation, distilleries generate dark brown color effluent (pH 3.0-4.5) of about 8-15 times by volume of the alcohol production, which is called "spent wash". It contains very high organic loadings Biological Oxygen Demand (BOD 40,000-60,000 mg/L) and Chemical Oxygen Demand (COD 80,000-1,20,000 mg/L)[4]. Due to very high organic loadings of the effluent, distillery sector has been identified as one of the highly water polluting category and grouped under "Red" category of highly polluting industries.

Various efforts are made to minimize pollution load by modification of fermentation and distillation processes. Fermentation processes, being practiced, are batch, fed batch or continuous type[1]. Atmospheric distillation and Multi Pressure (Vacuum) distillation processes are being used for distillation of alcohol. These processes optimize feed concentrations and product concentration vis a vis to minimize volume and organic loads of effluent. These modifications enabled to minimize effluent volume form about 15 times of the volume of alcohol production to about 8 times of the volume of alcohol production. However, this reduction in volume of effluent simultaneously leads to increase in organic loads of the effluent. In this study, we have changed the raw material for alcohol fermentation to minimize pollution load. Grains have been used as raw material in place of molasses, which contains about 65-70% starch[5]. This results in low volume of effluent generation with lesser organic loads (low BOD/COD values) and absence of Caramel in the effluent.

EXPERIMENTAL

Experiments were carried out in different sets of fermenters in batch fermentation mode. Molasses and Broken rice (grain) were used main raw materials as carbon source for fermentation of alcohol. Enzymes, α-amylase and amyloglucosidase, were used for hydrolysis of starch. The yeast, *S. cerevisiae*, culture was used for fermentation of sugar to alcohol.

Fermentation of Molasses

Raw material, molasses containing 42.50-44.00% total residual sugar, was diluted to 40^{o} brix (≈15% sugar) and sterilized. Fermenter containing initial diluted raw was inoculated from seed tank (yeast biomass 1-2 x 10^{6} cells/mL). Fermentation was carried out at 35-37°C. Samples were taken from sampling point to analyze specific gravity and alcohol concentration at different intervals.

Fermentation of grain

Raw material, grain contains about 64-78% starch, 7-14% proteins and other constituents as shown in Table 1. Grains (Broken rice) were cleaned, screened and grinded (milled) to powder. Then slurry was prepared for cooking by diluting it to about 3 time using water. Cooking was carried out in a jet cooker by direct stream injection at 115-120°C temperature. After cooking, slurring was cooled and liquefying and saccharifying enzymes (α-amylase 0.8 Kg/Kg of starch and Amyloglucosidase 1.0 Kg/Kg of starch) was added for liquefaction and saccharification[5,6]. Starch hydrolysis mechanism is shown in Fig. 1. After partial saccharification, it was transferred to fermenter and inoculated with yeast cells (conc. 1-2 x 10^6 cells/mL). During fermentation process further saccharification and fermentation take place simultaneously. Fermentation was carried out at 35-37°C. Samples were taken from sampling point to analyze specific gravity and alcohol concentration at different intervals.

Table 1: Average composition of grains on dry basis[5]

Grain	Starch (%)	Protein (%)	Fiber (%)	Others* (%)
Hard wheat	64	14	2	20
Soft wheat	69	10	2	19
Raw rice	78	7	0.2	14.8
Parboiled rice	77	9	0.2	13.8
Dent corn	72	10	2	16
Sorghum	71	13	2	14

***Oils, Minerals, Vitamins etc.**

$$(C_6H_{10}O_5)_n + nH_2O \xrightarrow{\alpha-amulase/Amyloglucodiase} n(C_6H_{12}O_6)$$

$$C_{12}H_{22}O_{11} + H_2O \xrightarrow{S.cerevisae} 2C_6H_{12}O_6$$

$$C_6H_{12}O_6 \xrightarrow{S.cerevisae} 2C_2H_5OH + 2CO_2$$

Fig. 1: Fermentation of starch to ethanol

After fermentation, fermented broth was settled to remove yeast sludge and fed to distillation unit, where alcohol is distilled and bottom residue of broth (spent wash) is taken out. Physio-chemical properties of spent wash (pH, residual sugar (RS), alcohol concentration, Total Suspended Solids (TSS), Total Dissolved Solids (TDS), BOD and COD) were measured.

RESULTS AND DISCUSSION

Kinetics of fermentations

Fermentation of both molasses and grains were carried out in batch fermentation mode. During fermentation specific gravity of fermentation medium decreases simultaneously with formation of ethanol due to its lower specific gravity (0.79 gm/mL). Decrease in specific gravity of fermentation broth during fermentation of molasses and grain to ethanol is shown in Figure 2(a) and 2(b) respectively, which reflect the synthesis of ethanol.

Degradation of starch to ethanol is express by first order kinetics[7] as follows:

$$y = A\left[1 - \exp\left(-t * \tau\right)\right] \qquad \dots(1)$$

where A is an arbitrary constant and τ is rate constant (hr^{-1}).

Equation 1 is fitted to experimental data as shown in Fig. 2. Experimental data are fitted quite well with correlation coefficient (R^2) ≈0.99. The rate constant (τ) for molasses based fermentation is 6.3 x 10^{-2} hr^{-1} to 9.2 x 10^{-2} hr^{-1} and that for grain based fermentation is 1.1 x 10^{-2} hr^{-1} to 1.3 x 10^{-2} hr^{-1}. Similar value of τ for fermentation of starch is reported[7].

At the end of fermentation, concentration of alcohol in fermented broth is 7-8% v/v from molasses and that is 12-14% from grain. The increase in alcohol percentage results due to high sugar content of raw material (grain). Further, mechanism of starch hydrolysis favors to feed high concentration of sugar. The tolerance limit of yeast is about 15-20% sugar. Thus molasses is diluted to maintain the desired sugar concentration. While starch is partially converted to sugars during liquefaction and fed to fermenter. Further saccharification and fermentation take place simultaneously. During fermentation, yeast biomass consumes sugar to produce alcohol. This causes decrease of sugar content, which is supplemented by further hydrolysis of starch to sugar. This enables to feed high concentration of sugar in fermentation, which in terms results more percentage of alcohol. Due to varying content of alcohol in fermented broth, volume of spent wash varies. The volume of spent wash is about 12-15 times of alcohol content for molasses based fermentation, while that is about 6-8 times of alcohol content for grain based fermentation.

The content of residual sugar at the end of fermentation is about 1.8-2.2%[8] for molasses based fermentation and that is about 0.2-0.3% for grain based fermentation. This leads to reduced organics content in the spent wash from grain based fermentation, which is discussed in the next section.

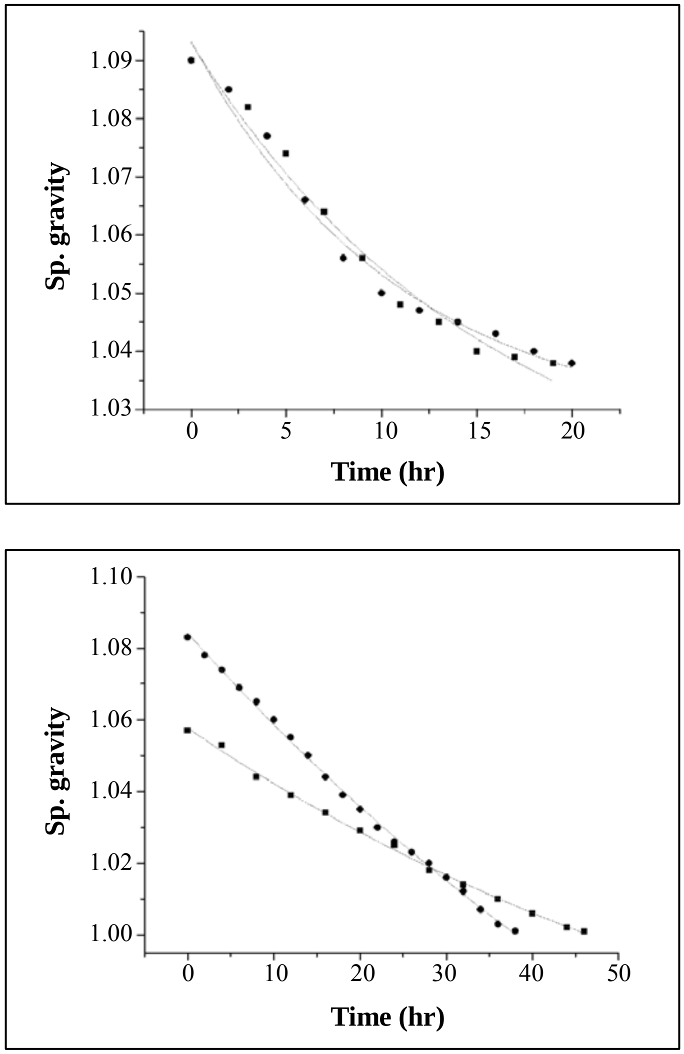

Fig. 2: Decrease in specific gravity of fermentation broth during batch fermentation of (a) molasses and (b) rice. Experimental data of two different sets are shown by symbols and lines represent fitted data using equation 1

Quality of effluents

Physio-chemical properties of spent wash (pH, Total Suspended Solids (TSS), Total Dissolved Solids (TDS), BOD and COD) generated from molasses and grain fermentation were measured. This is shown in Table 2. Average value of BOD and COD for molasses based spent wash is about 40,000 mg/L and 1,10,000 mg/L, respectively and that for grain based spent wash is about 30,000 mg/L and 75,000 mg/L, respectively. The BOD value is reduced about 19% and COD value is reduce about 30% for grain based fermentation as compare to molasses based fermentation. Also as the grains do not contain caramelize sugars, it does not import colors to spent wash as compare to molasses as raw material. These will ease the management of effluent from grain based fermentation process. Further various options for effluent management need to be modified as compare to that of molasses based fermentation.

Table 2: Quality of molasses and grain based distillery effluents

Parameters	Molasses based spent wash	Grain based spent wash
pH	5 ± 0.3	4 ± 0.3
TSS	7124 ± 673	38174 ± 15315
TDS	98358 ± 22065	13594 ± 3906
COD	107661 ± 40637	75532 ± 25780
BOD	39483 ± 22264	31888 ± 8976

CONCLUSION

We studied the changed raw material for fermentation of alcohol. We used grains instead of conventional molasses as carbon source. Saccharification and fermentation of starch to alcohol take place simultaneously, which enable to feed high sugar content for fermentation. This results higher concentration of alcohol in fermented broth (12-14%) for grain based fermentation as compare to 7-8% v/v alcohol for molasses. Thus, volume of spent wash is about 12-15 times of alcohol content for molasses based fermentation, while that is about 6-8 times of alcohol content for grain based fermentation. The content of residual sugar at the end of fermentation is about 1.8-2.2% for molasses based fermentation and that is about 0.2-0.3% for grain based fermentation. This leads to reduced organics content in the spent wash from grain based fermentation. The BOD value is reduced about 19% and COD value is reduced about 30% for grain based fermentation as compare to molasses based fermentation. Also, as the grains do not contain caramelize sugars, it does not import colors to spent wash as compare to molasses as raw material.

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