

STRATEGIES TO IMPROVE FERMENTATION YIELDS AT TRIANGLE ETHANOL PLANT IN 1999

RA PEREIRA AND PD SMITH

Triangle Limited, P/Bag 801, Triangle, Zimbabwe

Abstract

During the early part of 1999, Triangle Limited experienced problems at their Ethanol plant with low yeast populations and incomplete fermentations that resulted in very poor yields. Similar problems in 1990 were attributed to toxic levels of copper in one of the nutrients added in pre-fermentation (Madaree et al., 1991). However, in this case, laboratory analysis had indicated no abnormally high levels of copper or other metals.

In an effort to solve this problem and to improve fermentation yields the following strategies were implemented: -

- Food grade sulphuric acid was imported from South Africa to replace the sulphuric acid available locally. The preparation procedure for the yeast culture, which involves the addition of sulphuric acid, was modified to prevent the low pH previously obtained.
- An extra air blower was added to the plant and more holes drilled in the pre-fermenter sparge pipes to improve aeration of the yeast culture.
- A fed batch system of adding nutrients to the yeast culture was introduced to improve yeast propagation. The concept of fed batch cultures is introduced and the results of this implementation are presented.

The above modifications, allied to stringent sterilisation procedures and closer supervision have resulted in improved yields. In this paper actual plant data are presented to show the fermentation yields before and after these modifications.

Introduction

Ethanol Plant Feedstock

The feedstock for the Ethanol plant is mainly C-Molasses from the sugar factory, although some molasses is imported from the neighbouring factory at Hippo Valley.

Typically, molasses that is sent to the Ethanol plant has the properties indicated in Figure 1, below.

Molasses is diluted with water to form a mixture called mash, before it is suitable for use in fermentation. The fermentation

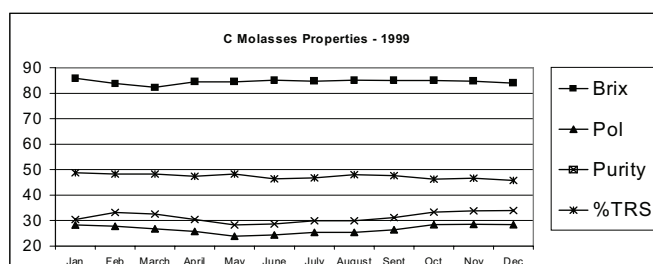


FIGURE 1 C Molasses properties, 1999.

process at Triangle is broken down into the following two phases:

Pre-fermentation

About 35m³ of mash is introduced into the sterilised pre-fermenter, and this is inoculated either with yeast from a previous batch, or if a new batch is being started, by a preparation of dry baker's yeast (*Saccharomyces cerevisiae*) mixed with chlorinated water. The pH of the mash and the yeast inoculum is controlled by the addition of sulphuric acid to prevent contamination of the pre-fermenter. Urea is also added to the mash, to provide sufficient nitrogen for the propagating yeast.

The pre-fermenters are continuously sparged with air, ensuring that the yeast has sufficient air for respiration, and that the contents of the pre-fermenter are well mixed.

Once pre-fermentation is complete, 4-5m³ of the yeast culture is gravity fed into the yeast holding tank and the remainder, about 35m³, drains to a main fermentation vat. This procedure is repeated until the yeast dies or is terminated.

Fermentation

In the main vat, the yeast culture is fed with mash, which ferments to produce ethanol. The concentration of ethanol at the end of fermentation is 8-10%v/v. In order to ensure a successful fermentation in the vats, it is important that the pre-fermenters produce a high population of viable yeast.

Previous work with problem fermentations

An investigation into fermentation problems experienced in 1990 yielded the following results (Madaree et al., 1991):

- Sugars, especially sucrose, were not utilised by the yeast, and there was no diversion to other end-products. The levels of bacteria and wild yeast contamination did not account for the poor pre-fermenter performance.
- The yeast was resilient to pHs as low as 1 and temperatures as high as 40°C. In fact, there seemed to be an increase in yeast viability as the pH dropped from 2 to 1.
- The cause of the incomplete fermentations was found to be toxic levels of copper in the diammonium phosphate, a nutrient that was used in pre-fermentation. It was determined that the copper was bound on the cell surface, preventing the inversion of sucrose to monosaccharides. The critical level of copper affecting ethanol yield was between 20-40ppm, depending on the size of the inoculum.
- It appeared that the concentration of copper in the yeast accumulated as the yeast was recycled.

Since commissioning the C Station in June 1994, fermentation yields have been low, as indicated in Table 1, below. Typically, the yield on TF (Total Fermentables) should be 59 and the yield on TRS (Total Reducing Sugars) should be about 55-56. (Madaree *et al.*, 1991)

Table 1. Fermentation Performance¹

Season	Fermentation Yield litres ethanol/100kg TRS	Fermentation Yield litres ethanol/100kg TF
94/95	52.6	57.5
95/96	52.8	57.9
96/97	50.8	55.6
97/98	50.9	55.7
98	45.9	50.5
99	49.9	53.8

Late in 1998, and continuing into 1999, the yeast culture was changed regularly because the fermentation yields decreased significantly, to approximately 30% of theoretical, within a few days of starting a new culture. It appeared that the cell populations in the pre-fermented yeast culture were too low to sustain fermentation in the main fermenters. However, brix measurements indicated that large quantities of sugars remained in the beer, after fermentation had ceased. (Madaree and Smith, 1999).

Although the fermentation yields improved in the latter part of the year, the initial source of the problem was not categorically identified. In fact, the poor yields were probably the result of a combination of factors. Consequently, the fermentation process was reviewed and various modifications, both in operating procedure and plant design, were made that resulted in improved fermentation yields.

Conditions affecting the growth of *S. cerevisiae*

The propagation of the yeast, *S. cerevisiae*, is affected by the following environmental conditions:

Temperature

The recommended operating temperature in the pre-fermenters, for *S. cerevisiae* is 28-32°C. At Triangle the pre-fermenters are operated at 32°C, as per the designer's original specification. (²Stegemann, 1979).

The growth rate of yeast approximately doubles for every 10°C rise in temperature until the optimal temperature is reached, after which the growth rate decreases and the organism eventually dies. Typically, thermal death rate of yeast is more sensitive to temperature changes than the growth rate of yeast. (Shuler and Kargi, 1992).

pH

Hydrogen ion concentration affects the microbial growth rate, although there is generally an acceptable pH range around the optima of 1-2 pH units. During fermentation, pH may vary for a number of reasons: nature of nitrogen source, production of organic acids or bases, evolution of CO₂ etc. (Shuler and Kargi, 1992). The pH of the pre-fermenters is controlled at 4.5, and is stable during the course of the pre-fermentation, unless more mash is added to the culture.

Dissolved oxygen concentration

Oxygen is an important substrate in the pre-fermentation process and may be a limiting factor, since oxygen is sparingly soluble in water. There is a *critical oxygen concentration*, different for each culture, above which the yeast growth rate is independent of the dissolved oxygen concentration. For the propagation of *S. cerevisiae* this concentration is about 0.7ppm (Shuler and Kargi, 1992). Not only does a low oxygen concentration result in low growth rate, but there is the danger of ethanol production rather than the required yeast propagation, as the conditions become more anaerobic.

Substrate Concentration

Molasses is the sole source of glucose for the culture and both high and low concentrations of this substrate would inhibit the propagation of the yeast. At low concentrations there would be insufficient nutrient for growth and at high concentrations, even under aerobic conditions, ethanol production takes place. At high substrate concentrations, the synthesis of respiratory enzymes is inhibited by the catabolic products of glucose. This metabolic regulation of yeast by the substrate is known as the *Crabtree effect* (Crabtree, 1929; Phaff *et al.*, 1978).

D'Amore *et al.* have also reported that there is an increase of intracellular ethanol in response to an increase in the osmotic pressure, such as that caused by an increase in glucose concentration. The effect of intracellular ethanol is to decrease the growth rate of the yeast. (Stewart *et al.*, 1982)

Presence of toxins or bacteria

Toxins may affect cells in various ways, e.g. altered cell morphology, altered cell metabolism, bacteriostasis (cells are viable but do not multiply) or lethality.

Heavy metals are toxic at concentrations varying from 0.4-600ppm. (Madaree *et al.*, 1991)

At low pH, lactic and acetic acid are inhibitory to the growth of yeast. (Phaff *et al.*, 1978) The presence of lactic acid is also an indication of contaminant bacteria. (Madaree *et al.*, 1991).

At high concentrations, usually above 8-10%, ethanol ceases to be merely inhibitory, and becomes poisonous to the yeast (Stewart *et al.*, 1982). Thus, rather than merely halting propagation, viable cells are now killed.

Operational and Plant Modifications

Poor yeast propagation in the pre-fermenters was a catalyst for Triangle to re-visit the operational procedures, and the plant design itself, to see where improvements could be made. The

¹ Note: In 1998 the financial year-end changed to December.

² Design report, Stegemann 1979.

basis for any modifications were the environmental factors outlined in the previous chapter, and in some cases a strategy addressed several of these factors.

Temperature control of the pre-fermenters is carried out by cooling water that flows down the side of the vessels, and this procedure has been found to be satisfactory, except if there is a breakdown in one of the cooling tower fans, which is an extremely rare occurrence. During the factory off-crop when the factory cooling towers are being maintained, the fermentation station is supplied with cooling water from the power station cooling towers. Occasionally, the cooling water from the power station towers reaches 35°C and the pre-fermenters run at higher temperatures. However, this problem is generally transitory and there have been no apparent negative affects on yeast propagation.

The following were modifications carried out:

Quality and quantity of H₂SO₄ added to the pre-fermenters

In February 1999, a report from the Technical Management Department (TMD), of Tongaat-Hulett in Durban indicated that the amount of sulphuric acid added to a new yeast culture by Triangle's operators would give a pH reading, in the TMD lab, of one unit less than that obtained on the plant. (Madaree and Smith, 1999). There was also a concern at Triangle that the operators were overshooting the pH target because of the addition of large amounts of acid. This procedure was modified to ensure that the pH meters were calibrated before starting a yeast culture and that the acid was diluted and added in small quantities, followed by vigorous stirring and frequent measurement of the pH, to ensure that the target pH of 2.5 was not exceeded. Supervision by the plant overseers is obligatory for this procedure.

Analysis by TMD in December 1998 also indicated that the heavy metal content of the sulphuric acid used by Triangle had increased since the acid was last analysed in 1989, as indicated in Table 2. (Madaree, 1989).

Table 2. Toxic Metal Analysis of H₂SO₄

Element	1989	1998
Copper (mg/kg)	5.4	11.3
Lead (mg/kg)	0.7	33.0
Chromium (mg/kg)	5.3	18.2
Arsenic (µg/kg)	18.0	10.6

Although sulphuric acid is significantly diluted in the medium, Triangle have since changed suppliers and now obtain the acid from a source that claims much lower levels of heavy metal contamination.

Aeration of the pre-fermenters

Originally, the single air blower, a Robuschi RVT 130, that was installed at the ethanol plant was adequately sized to supply one pre-fermenter. However, recently it had sometimes been necessary to divert some of this air to the molasses tank farm, to cool the molasses tanks or reduce the foam levels in the

tanks. Obviously, this situation was not ideal as it reduced the amount of air available to the pre-fermenters. In June 1999, an additional unit, also a Robuschi RVT 130, was installed in the ethanol plant. This has ensured that one blower is dedicated to pre-fermentation and the other blower serves the molasses tank farm.

Furthermore, it was found that the amount of air supplied to the pre-fermenters (PF107, PF108 and PF109), as measured by rotameters, varied for each pre-fermenter, with only PF109 receiving enough air, approximately 1000m³/hr. Upon investigating, it was found that there were fewer holes drilled in the sparge pipes of the pre-fermenters PF108 and PF107, than in PF109. In addition, the sparge pipes in PF108 were shorter than those in the other pre-fermenters. During the annual shutdown in June 1999, extra holes were drilled in the sparge pipes of PF108 and PF107, and the sparge piping in PF108 was extended to match the arrangements in the other pre-fermenters.

Air filters, that prevent bacterial contamination of the air, are now routinely cleaned on a weekly basis.

Optimising the addition of urea

In December 1999, a study was initiated to determine the optimal amount of urea needed, as a nutrient, by the yeast culture in the pre-fermenters. (This study was conducted after implementing the fed-batch method of operating the pre-fermenters. The fed-batch method is discussed later in this paper.) The parameters used in this investigation were:

- Final cell population
- Final viability of the cells
- Final Ethanol %
- Final Brix

A preliminary report concluded that the optimum range for the amount of urea to be added, to ensure the best results in each of the four parameters, was 19-32kg. However, the sample size was small and the results cannot be considered to be conclusive. Nevertheless, the present operating procedure calls for addition of 20kg of urea, which falls into the proposed optimal range. (Mubako, 2000).

Modifications of operating procedures

Since 1980, when the Ethanol plant was commissioned, many of the existing procedures have been lost or become obsolete as the process has been altered e.g. change in feedstock from juice, A and B molasses to C molasses. New procedures have been written for starting a yeast culture, sterilising the vessels and for the operation and monitoring of the vats and pre-fermenters.

A possible cause of the poor fermentation results may have been insufficient yeast inoculation of the pre-fermenters, caused by a low culture level in the previous pre-fermenter. Since the tops of the pre-fermenters and the yeast holding tank are at the same level, a low level of yeast culture in a pre-fermenter translated into a smaller charge in the yeast holding tank. This problem was attributed to measuring mash charge to the pre-fermenters through an unreliable batch weigher, which resulted

in inconsistent batch sizes. The mash charge is now measured by a flowmeter, which has resulted in more favourable batch sizes. However, it is equally likely that the smaller batch sizes were a result of operator error and the operators are required to check the level of each batch. The plant overseers monitor the batch levels routinely (³ personal communication).

TMD reported that the water to yeast ratio used to start a new yeast culture was 5.3 instead of the recommended 2.5. (Madaree and Smith, 1999). This procedure has been modified and a water to yeast ratio of 3 has been implemented.

An area of concern has been the possibility of errors in the laboratory analysis of samples from the ethanol plant. This analysis is the basis of yield calculations and is generally the first indication of problems at the ethanol plant. In January 2000, TMD reported that the Triangle laboratory had been over-estimating sugars in the molasses, during the 1999 season, by 2.5 units. This equated to an underestimation of fermentation yield of about 3.5 units. (Madaree and Smith, 2000). This affected the fermentation yields for the whole year, and partially explains the poor results.

The analysis of ethanol loss in stillage has also been uncertain but, with the recent acquisition of a Gas Liquid Chromatograph and an appropriate column, this problem should be solved.

Training of the laboratory staff has been carried out on a regular basis and this shall help to ensure the reliability of yield figures and the accurate measurement of ethanol content.

Fermentation feed-rate profile

The ethanol plant at Triangle was first commissioned to accept a combination of juice, A and B molasses. Since the installation of the C Station in 1994, the ethanol plant has only received C molasses, which contains a lower proportion of sucrose, and a higher proportion of non-fermentable dissolved substances than the previous feedstock. Thus, it was postulated that the original strategy for feeding the yeast in the pre-fermenters, using a single batch of mash, might not be optimal and a number of other strategies have been tried (Modak and Lim, 1987).

In the pre-fermenters, the yeast should be allowed to respire aerobically, so as to release the maximum amount of energy, which is needed for growth and propagation. It is also important to minimise the Crabtree effect, by limiting the substrate concentration in the pre-fermenters. Maintaining a lower mash concentration would also reduce the osmotic pressure, thus allowing any intracellular ethanol to be released (Stewart et al, 1982). Furthermore, the amount of air available to the mash must always be greater than the critical oxygen concentration.

Consequently, the ideal scenario would be to gradually feed the yeast throughout the 8 – 9 hour pre-fermentation, and thus allow the culture to continuously metabolise the incoming substrate (Modak et al, 1986). However, due to pumping limitations, this is not possible, and a good approximation is to feed the yeast in several discrete batches, usually two or three, spaced throughout the course of pre-fermentation. This gradual

feeding has the added advantage that the pre-fermenter would be operating for a large part of the fermentation time below its normal full level, and thus the blowers would be able to work against a reduced pressure head, and would thus be able to deliver air at a faster rate. More vigorous aeration would also result in better mixing, thus eliminating local concentration differences.

Over the past three seasons, fed batch fermentation has been used intermittently, in the pre-fermenters (1996 and 1997) and the main fermenters (1996) in order to stretch a particular batch for CO₂ production, and the results have been promising, as shown in Table 3, below. It was interesting to note that, during this period, the mash was not aerated until the pre-fermenter was full and, initially, the yeast culture fermented anaerobically. Consequently, the growth rate of the yeast would have been low or, possibly, non-existent.

Table 3. Fed-Batch Results (1996-1998).

Year	1996	1996	1997	1997	1998
Ferm.	Conv.	F.B.	Conv.	F.B.	Conv.
Yield	48.2	53.7	50.4	51.1	47.0

However, as shown in Figure 2, these results represent a relatively small number of batches, all run within a narrow time, and as such do not fairly compare the two scenarios.

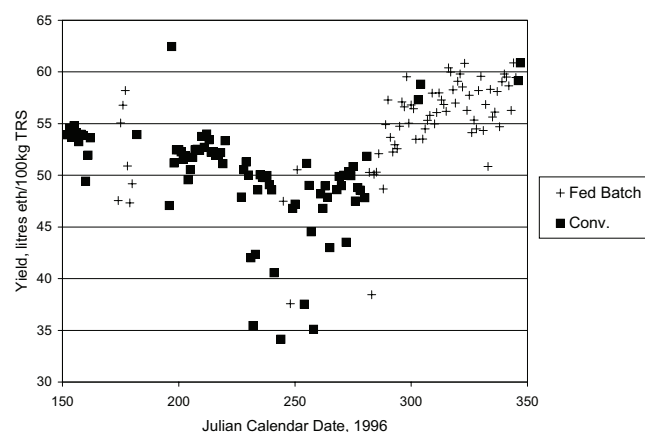


FIGURE 2. Results of fed batch fermentations, 1996.

It is also interesting to note that there were several conventional fermentations that also achieved good results towards the end of the season. This was thought to be due to the improvement in the quality and viability of the yeast, which was brought about by adaptation to the preceding fed batch fermentations.

Table 4. Results of Fed-Batch Experiments.

Strategy	Units Added at Each Time				
	0hr	3hr	4hr	5hr	6hr
1	400				
2	200		200		
3	200				200
4	200		100		100
5	134	133			133
6	200	100		100	

³ C. Mapfaira, Ethanol Plant Supervisor

Throughout the second half of the 1999 season, this mechanism was tried consistently, with a number of different strategies. Pre-fermenters are usually charged with about 400 units of molasses, which equates to about 35 m³ of mash. Table 4, shows the times at which molasses was fed and the amount used each time. In these cases, maximum aeration was implemented during the full course of the fed-batch fermentation.

Results of Fed-Batch Experiments

The results of these experiments have been very promising, with a notable increase in the fermentation yields. These are presented in Table 5, below.

Table 5. Ethanol Yields with Different Feeding Profiles.

Feed Profile Molasses Units (time)	Strategy	Ethanol Yield l/100kg TRS	No. of Batches
400(0h)	1	47.7	363
200(0h)+200(4h)	2	51.6	159
200(0h)+200(6h)	3	52.0	30
200(0h)+100(4h)+100(6h)	4	54.8	113
134(0h)+133(3h)+133(6h)	5	54.5	8
200(0h)+100(3h)+100(5h)	6	54.2	7

As can be seen, method 4 gave the best results, and this was achieved with a statistically significant number of batches. A possible reason for this was because method 4 provided the best trade-off between the several objectives for this problem, namely:

- For best mixing, the pre-fermenter should be at a low level for as long as possible.
- To avoid substrate inhibition, the mash concentration should not be increased too suddenly.
- To achieve maximum utilization of sugars, the culture must be left for sufficient time for them all to be used, but not so long that the yeast runs out of substrate.

Overall Results

The overall result of the improvements made during the 1999 season can be seen most clearly in figures 3 and 4, below.

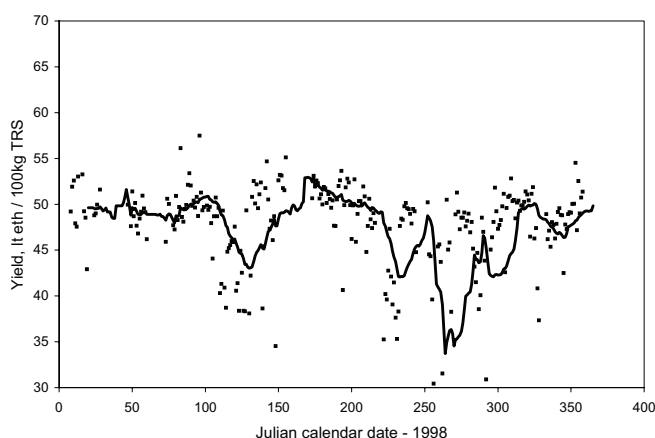


FIGURE 3. Results of conventional fermentations, 1998.

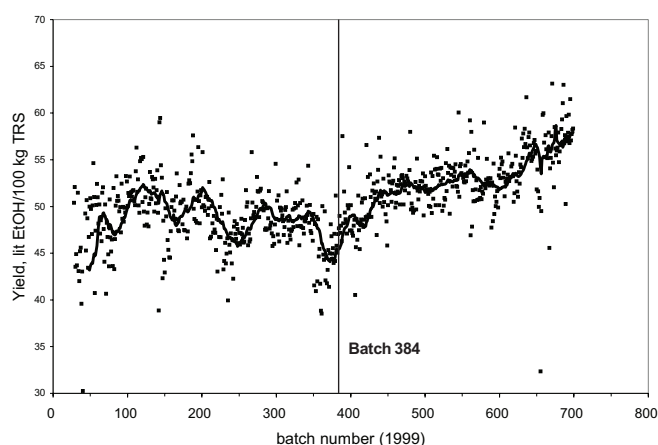


FIGURE 4. Results of fed batch fermentations, 1999.

Another valuable observation from these two figures is the ability of the yeast to adapt to a new feeding regime, or manner of treatment. In figure 4, during the 1999 season, from batch number 384 (when fed batch fermentations and other improvements were implemented) to the end of the season, there is a steady increase in fermentation yields. This has been attributed to the adaptation of the yeast to the new feeding regimes, and increased aeration. A similar effect was observed during the 1996 season, as shown in Figure 2.

The final result of these modifications can be summarised by comparing the average yield achieved in the first part of 1999, 47.7, to that achieved after the modifications were effected, of 52.9. This was an increase of more than five litres per 100kg TRS, a 10% increase. Considering the fact that the fermentation yield increased throughout the latter part of 1999, this is a significant result.

Conclusion

In this paper, the problems that have faced the Triangle Ethanol Plant, over the past 10 years, have been introduced. Several investigations have already been done to find the reasons for incomplete fermentations, or low yeast viability. During the 1999 season, each of these remedies was revisited, so that past mistakes would be avoided. In addition, a number of modifications have been implemented to improve fermentation yields. More stringent operating techniques have been devised, and these are now rigorously applied. Aeration has been enhanced by the installation of a second blower unit. Finally, a fed batch system for pre-fermenter feeding has been implemented. Several strategies were tried, until an optimum method was found. The results from these modifications have been striking, a more than 10% increase in ethanol yield.

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