Recent technology developments will allow process improvements to be introduced into the biofuels industry driving yield, and hence, plant profitability higher that the currently expected yield baseline results

Introducing the first commercial genetically enhanced yeasts

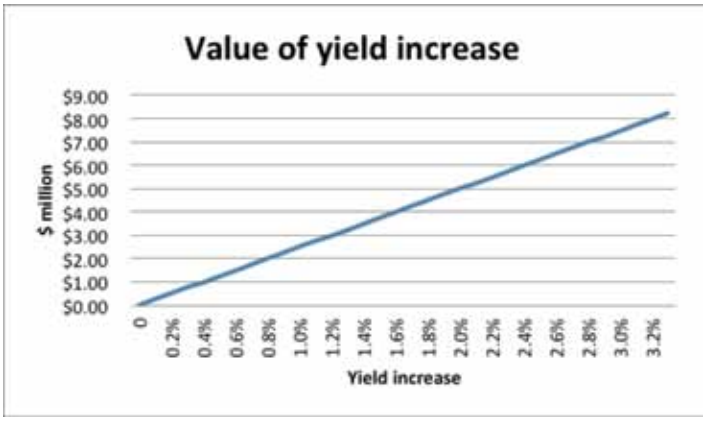
The industry has undergone unprecedented growth over that last five years. However with that growth came major questions in regards to using feedstock for fuel instead of feed. This myth is largely untrue but criticisms coupled with legislative pressure and uncontrollable weather conditions have brought this industry to a crossroads.

The theory used to be to make as much alcohol as possible and not worry too much about the efficiency and yield. Now with the profitability and margins being tested at every turn, it is time for plants to look into various ways to maximise the system and to get as much yield out of the feedstock as possible.

One of the ways to look at the overall process is to determine which feedstock is needed to run efficiently. Also looking at various parts of the production facility and see where improvements can be made.

One of the most important areas in fermentation - this is the only area where the alcohol will be produced. It is clear to anyone involved in the production of ethanol that current high prices of grains worldwide are creating a major issue in respect of profitability.

What implication does this have on fermentation and the technology surrounding it?



The value of percentage yield increase in a 100 million gallon plant

By definition, yield increase is 'increasing the quantity of final product produced from the process without increasing the quantity of feedstock utilised.' The increased revenue (or savings) from increasing yield directly impacts the bottom line of a production plant as no additional processing or fixed costs are required.

There is a significant increase in bottom line benefits as modest yield improvements are increased.

Improving the fermentation process

The process is already very efficient and a well controlled plant is capable of running at over 92% efficiency, this is a respectable number in any industry. The problem is more eliminating yield loss or improving cost efficiency rather than improving the basic process. Of course there are always new technologies that look to novel methods to increase efficiency.

A well controlled plant will monitor its process through key performance indicators (KPI's) and identify process variability or 'lack of control'. There are a number of areas in a biofuels process that can be identified as negatively affecting the efficiency of the process. In simple terms there are three possible key areas of yield loss

1. Fermentable sugars are not fully extracted from the grain (starch)
2. Fermentable sugars are not fully converted to alcohol
3. Alcohol is produced and then 'lost' post fermentation

Areas 1 & 3 are not directly related to fermentation and hence will not be discussed in this article. But the second area is absolutely key to improving the process performance.

What negatively affects fermentation?

In simple terms, the yeast requires certain conditions to perform effectively. Basically there are only four situations that can create potential issues

1. The environment is lacking in a component
2. There is something present that is stressing the yeast
3. There is something present that is competing for the basic feedstock
4. There is a condition that is killing the yeast

1) Yeasts nutritional requirements

There are a number of key nutrients that are critical to the yeast performance for many reasons, but there are also key aspects of the feedstock and process parameters that also fall into the 'nutritional' area. For instance, if the glucose level falls below 1% then this can naturally cause a stalling of the fermentation. In addition, the fermentation process is anaerobic and without the presence of free oxygen, components such as sterols and unsaturated fatty acids cannot be metabolised. Both these compounds provide components of a healthy yeast cell wall and membrane. A restriction of their availability will reduce the exponential growth phase by restriction of the budding percentage.

Typical criteria for yeast nutritional requirements

Category	Description	Comments
Carbohydrates	Glucose, maltose, maltotriose	These are the only sugars the yeast can utilise listed in order of preference, without them fermentation and growth stops
Nitrogen	Small chain peptides, amino acids, ammonia, urea	Amino acids are required to allow the yeast to produce proteins & enzymes and hence critical to growth and fermentation. The yeast can either take in these building blocks or assimilate them from a raw nitrogen source
Other major components	Phosphorus, sulphur and oxygen	
Macro nutrients	Potassium, calcium, iron, manganese, chloride, magnesium and zinc	Most of these are present in grain mash, with slightly varying levels depending on the type of grain and environmental conditions during season
Micro Nutrients	Cobalt, boron, cadmium, chromium, iodine, molybdenum, nickel, and vanadium	Most of these are present in grain mash, with slightly varying levels depending on the type of grain and environmental conditions during season
Vitamins	Biotin, pantothenic acid, Inositol, thiamin, nicotinic acid and pyridoxine	

Due to the potential of mash components changing from week to week, through differing sources of grain, a number of the most consistently controlled production processes use a commercial yeast food to ensure that critical nutritional components are always present. Generally these products are cost effective and are justified by the more consistent results.

It is clear that in the respect of nitrogen, which after carbohydrates is considered the most important nutritional component, there is significant data to show the benefit of supplying suitable amino acids as opposed to relying on other chemicals such as ammonia or urea. Based on this a significant number of facilities will use a protease to supply amino acids and small di and tripeptides through the breakdown of proteins present in the mash. The end result here is generally a spike in the metabolic activity of the yeast combined with a slightly lower requirement of other nitrogen sources. Please note with certain proteases a slight yield improvement has been identified and is generally linked to the breakdown of the protein structure freeing up bound starch that can then be used either in the current fermenter or through the backset recycle in following fermentations.

2) 'Something is stressing the yeast'

This can happen from a number of sources. Some of them include typical components from feedstock or feedstock preparation, chemicals from processing/ cleaning materials, byproducts from contaminants or products recycled from previous batches.

In all of these cases the stressor exerts a negative effect on the yeast through chemical or metabolic impacts. The most common of these are as follows:

1. Organic acids (lactic and acetic acids) produced by contaminating bacteria or wild yeasts
2. Sodium residue from cleaning cycles. In zero discharge plants, all waste caustic (sodium hydroxide) has to be returned to the process or removed off site through a high cost approved method.

Of course there are a number of other components that can directly cause issues to the fermentation, but usually the high mash volumes and associated dilution in the fermentor can limit the effect. The organic acid issues are generally produced by the presence of Gram positive lactic acid bacteria (LAB). These organisms are very prevalent in the environment and are very difficult to eliminate from the process. A common methodology is to use a small maintenance dose of antibiotics in both the propagation as well as the fermentation. The most common treatment is that of Virginiamycin, Penicillin or a blend of antibiotics. The aim here is to eliminate or at least restrict the growth cycle of the bacteria thus eliminating the production of the organic acids.

The bacteria have a dual impact in as much that it impacts both situations 2 & 3. Initially the bacteria consume the same nutritional requirements as the yeast and this has a direct relationship to yield loss in the following ways:

- For every molecule of lactic acid that is produced is a loss of one molecule of ethanol
- For every 90 grams of lactic made, 46 grams of ethanol could have been made
- Likewise from acetic acid, for every 60 grams of acetic acid made, 46 grams of ethanol could have been made.

In addition, the stress impact of the presence of these organic acids is even more dramatic. In practical terms if the level of contamination of LAB is in the region of 106 cells per ml. it is reasonable to anticipate a reduction in alcohol production of up to 1-2% alcohol by weight of the final alcohol content at fermenter drop. In a 750,000 gallons (2,839,000 liters) fermentor this would equate to a loss of approximately $25,000 – $50,000 (€19,000-€37,000). Take this times the number of fermentors run and this loss adds up very quickly.

Other treatment options can include non-antibiotic options that allow microbial control and a potential premium in respect of DDGS pricing. Therefore multiple options are being explored to achieve this and the three most common choices are:

1. Hop acids – known to have a bacteriostatic effect is probably the most common Non-antibiotic option
2. Generated chlorine dioxide – a very powerful reducing agent that will kill bacteria at levels as low as 2-5ppm but yeast can survive up

to 50ppm without serious effect. The product has a very short half life and has very little residual effect, but very effective immediate kill

3. Stabilised chlorine dioxide – This utilises the same chemical as the generated version but relies on the pH drop caused by the production of organic acid to release the chlorine dioxide thus creating a kill of the bacteria. This product has a good kill effect later in the fermentation but has almost no immediate kill as the product has not been activated

Lallemand Biofuels and Distilled Spirits has carried out testing on a prototype methodology to pre-activate a stabilised chlorine dioxide solution to allow a very effective blend of options 2 & 3 giving a very effective immediate kill along with a good residual late fermentation effect. Initial testing indicates an effective control of bacteria in fermentation, along with the ability to reduce dosing levels once the system stabilises after the first 10-14 days of treatment. Based on this dosing reduction a cost benefit can be seen.

Another thing that could stress yeast would be the level of sodium throughout fermentation. It has been seen that stresses are synergistic and in general times of high stress, physical parameters or chemical stress, the impact of sodium can be seen at much lower concentrations than in times of low stress. Generally it is accepted that sodium level between 800-2000 ppm can cause poor fermentations.

The good thing in respect of sodium is that the vast majority of it originates from the use of caustic soda as a cleaning agent. Therefore good manufacturing practices and control can limit the sodium levels in the process.

2) 'Something is killing the yeast'

In most cases, out of control physical parameters are more likely to kill the yeast than stressors which normally only inhibit the fermentation.

The two main parameters that can cause major fermentation issues are temperature and pH. The temperature can have an impact on both the enzymatic breakdown and the yeast performance. Remember that enzymes and yeast are made of proteins and at higher temperatures, proteins can become denatured. This will affect this functionality of them and is some cases, the denaturing is irreversible. Although pH can affect the yeast, it is unlikely to kill it. However it can have a devastating effect on the enzymes.

The yeast fermentation is an exothermic reaction generating heat, therefore good process control and heat exchange in the early stages of fermentation (first 24 hours) where the yeast is most active is required. Typically most heat issues occur during this early period, and rapid response to issues is needed to avoid significant losses. Typical responses can include a re-pitching of yeast and potentially additional enzymes and nutrient package to restart a failed fermentation.

So far we have addressed the potential for yield loss through fermentation management, but there are also options of ensuring the most effective choice of yeasts. Practically speaking the ability of the yeast to ferment sugars to alcohol is generic across all Saccharomyces cerevisiae strains including ordinary baking strains through to specialty distilling or wine yeasts. The use of specific distilling strains can significantly affect the efficiency of fermentations especially where differing feedstocks are being compared.

For instance, where sugar based fermentations are being compared to starch based feedstocks, the fermentation and thus the available sugar profiles are very different. The yeasts have a great preference for monosaccharides (glucose or fructose) over disaccharides (maltose) or trisaccharides and in starch based fermentations this sugar profile is managed through enzyme optimization.

In sugar based fermentations, the mixture can contain significant levels of sucrose, the yeast produces an enzyme called invertase that splits the sucrose into glucose and fructose. Traditional thinking was that high invertase levels would result in good fermentations however work has demonstrated that although low invertase does not result in effective fermentations, mid range invertase production produce the most effective sugar based fermentations by reducing osmotic stress on the yeast through a slower release of monomers from the sucrose.

Choosing the right yeast

There are many varieties of yeast so therefore, the correct choice of yeast strain needs to be matched to fermentation conditions and feedstock choice. Some yeasts handle high stress conditions better than others, and some yeasts are better suited to specific substrates suchas starch vs. sugar.

Recent developments in yeast technology have resulted in the first commercial genetically enhanced yeasts that create differentiated benefits compared to typical yeasts being used in the industry.

The first version of this yeast, TransFerm 1.0 expresses its own glucoamylase (GA) supplementing the commercial enzymes added to the process. In commercial fermentations, this yeast has enabled reductions of GA of between 50-75%. This creates a significant cost benefit to a plant.. This yeast has regulatory compliance in the United States through a Generally Recognized as Safe designation as well as being listed in the definition of the Association of American Feed Control Officials for the inclusion of Distillers Dried Grains (DDG)'

The next version of the this yeast is expected to be released during 2013, and initial testing has shown in addition to glucoamylase reduction, a 3-4% yield improvement has been shown in both laboratory and pilot plant tests

Looking forward to the next 18 months to 2 years developments, it is clear that technology is going to be developed and introduced to the industry that will drive yield potential beyond the currently expected norm. If the new yeasts can indeed demonstrate a 3-3.5% yield improvement this would create an overall benefit in excess of $8 million per annum for a 100 million gallon plant

However in some regions of the world, yield is not always the key driver. For instance, in India, the high level of drought areas and limited water resources, increasing the fermentation and process efficiency to allow higher final alcohol content creates significant water utilisation. In one plant increasing the alcohol by 3-4% alcohol by volume resulted in the saving of 400,000 liters (100,000 gallons) of water per day. In addition most of the Indian plants are not zero discharge and this resulted in a corresponding reduction in effluent discharge per day matching the water savings.

Therefore to conclude, significant process improvements can be achieved through good manufacturing practices covering

1. Hygienic control
2. Fermentation management
3. Yeast handling practices
4. Yeast strain selection. ●

For more information:
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