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Masse, Julie, Shu, Li, Jegatheesan, Veeriah, Gros, Jean-Bernard and Phong, Diep Dinh 2013, Variations in the physical and biochemical properties of sugarcane juice before and after microfiltration, in Solutions to environmental challenges through innovations in research, Asiatech Publishers Inc., New Delhi, India, pp.121-142.

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Variations in the physical and biochemical properties of sugarcane juice before and after microfiltration

Julie Masse, Li Shu, Veeriah Jegatheesan, Jean-Bernard Gros and Diep Dinh Phong

1. INTRODUCTION

The sugarcane (or Saccharum spp. hybrids) is a tropical plant, which can grow to 2.5 to 6.0 meters. It was found in New-Guinea, in the Pacific and in Indian Ocean islands to Malaysia. Sugarcane needs an environment where the temperatures do not drop below 20°C and it is easy to cultivate sugarcane if the climate is suitable. The biggest producers of sugarcane are Brazil, India, China and Australia. Each year more than 1000 millions tonnes are produced from those countries. The sugarcane can be used for the following purposes: (i) the sugarcane juice for the production of sugar; (ii) the bagasse is burnt to produce energy; it can also be used to make paper; (iii) the residue for the production of alcohol; and (iv) the sugarcane also serves as bio carburant.

Australia, especially the Northern part has the suitable climate for the sugarcane cultivation. The North East of Australia, the Queensland region has a tropical climate which is perfect for this plant. The sugarcane is mainly constituted of fiber; the energy is kept in the form of carbohydrate and more specifically the sucrose is stocked in the vacuole. However, why the plant synthesizes sucrose and stores it is not fully understood. Nevertheless, plants need to control the accumulation and the degradation of sucrose very strictly (Foyer et al., 1997); the plants use a set of enzymes which also regulate the rate of fructose and glucose in the plants but the exact process is not well known. The enzymes which can interact directly with the sucrose are the invertase, the sucrose synthase and glycolysis enzymes. Glycolysis enzymes react with glucose-6 phosphate and fructose-6 phosphate, although the rate of sucrose degradation will increase with increasing glycolysis. Another very important enzyme is sucrose-phosphate synthase,
which synthesizes sucrose-phosphate, which is immediately cleaved by sucrose-phosphate phosphatase to sucrose.

To obtain the crystallized sugar, it is necessary to extract the sugarcane juice from sugarcane. The process consists of several steps and during this period the spoilage of sugarcane juice due to the contamination of microorganisms as well as degradation of sucrose can occur. All these phenomena can change the taste, the color and other appearance of sugar cane juice and may eventually lead to rejection of juice from the production of sugar. Thus a specific interest in finding solution to reduce these problems exists and one of the solutions is utilizing a micro- or an ultra filtration membrane to filter the sugarcane juice.

2. BACKGROUND OF SUGAR PRODUCTION

2.1. Sugarcane juice production

The sugarcane juice production is continuous (Chou, 2000); during six month of each year (from July to December in Australia), the sugarcanes are cut and carried to sugar mills for processing. During the season, each week the best 5% of the crop will be harvested. The canes are cut closer to the ground because the sugar concentration is higher at the bottom of plant. The sugarcane stalks are loaded onto conveyor belts and subjected to hot water sprays to remove the dirt. Then, a rotating knife cut them into very short pieces. The extraction of the juice can be accomplished in one of the two ways: diffusion or milling. For the diffusion method, the cut stalks are dissolved in hot water or lime juice. In the milling process, the stalks are passed under several successive heavy rollers, which squeeze the juice out of the cane pulps. Water is sprayed throughout the process to facilitate the dissolution of the juice. Usually the mills use both extraction methods and mix the juice produced by both methods.

Normally in sugarcane, the juice is heated and then limed. The calcium-phosphate flocs that form precipitate in large clarifier tanks. The reason lime is added is two-fold: to neutralize acids and to form the calcium-phosphate flocs. Flocculants are added to increase the molecular weight of the flocs and improve settling in the clarifier. Once the mud is filtered out, a pale yellow liquid called thin juice is left out. The juice is pumped into an evaporator which heat it until the water dissipates and the syrup remains. The clear juice has probably only 15% sugar content but saturated sugar liquor, required before crystallization can occur, will have a sugar content that is closer to 80%.

The syrup is concentrated through several stages of vacuum boiling. Eventually, the sugar crystallizes out of the syrup, creating a substance called massecuite. The massecuite is poured into a centrifuge to further separate the raw sugar crystals from the syrup. In a raw sugar factory three boiling are generally conducted. The first boiling produces the
best sugar which is sent to store. The second boiling takes longer and the retention time in the crystallizer is also longer if a reasonable crystal size is to be achieved. Some factories re-melt the second sugar to provide part of the first boiling feedstock. The third boiling takes longer than the second boiling to crystallize. The sugar is usually used as seed for second boiling and the rest is re-melted. Crystals are controlled several times during the process to check the size and the form.

The final product which is raw sugar looks rather like the soft brown sugar found in domestic kitchens. It could be used like that, but usually it gets dirty in storage and has a distinctive taste, therefore it is normally refined when it exported to other places where it is eventually consumed. Additionally, this produces a sweet by-production which is called molasses. This is usually turned into a cattle food or is sent to a distillery where it can be used for the production of alcohol.

2.2. Microorganisms in sugarcane juice

Microorganisms are present in the outer surface of sugarcanes. Once the sugarcanes are cut microorganisms can grow in the cane (Yusof et al., 1999) and therefore during the step of crushing the sugarcane, they can infect the raw sugarcane juice. The majority of microorganisms found in the sugarcane juice are mould, Penicilliun and Aspergillus, yeast, Saccharomyces and Candida and bacteria Lactobacillus Acetobacter and Leuconostoc. For example, Sugarcane juice obtained from first and fifth internode of two different varieties of sugarcane showed the presence of diazotrophs in the order of $0.9 \times 10^2$ to $2 \times 10^4$ microorganisms per gram of sugarcane stem. These microorganisms use organic acids found in the sugarcane juice as energy source. They also use free glucose present in the aerial part of the sugarcane (de Bellone and Bellone, 2006).

The most important change to the juice is carried out by the bacteria as they produce acids by fermentation which involves the decrease in pH and the degradation of sucrose. Microorganisms can also use sucrose to produce polysaccharides like dextran.

2.3. Proteins in sugarcane juice

When the sugarcane is crushed, all the proteins stored in it are released to the raw sugarcane juice. Dry solids of sugarcane juice contain 0.5% protein. They can be a source of amino acids for microorganisms to entail their growth. They can also involve the browning of juice when they react with reducing sugar in the Maillard reaction (Sensidone et al., 1999). The first step is illustrated in Figure 1, in which glucose reacts with NH$_2$ extremity of an amino acid in a step of dehydration and in the subsequent steps, the reaction goes on until the formation of mellanoids compounds that are responsible for the brown color. By reducing the quantities of proteins and reducing sugar, the browning can be better controlled. However, Maillard reactions are of more concern in the late
evaporators and vacuum crystal pans.

2.4. Enzymes in sugarcane juice

Several enzymes can have an effect on the sucrose concentration (Hatch et al., 1963; Gillian and William, 2006), notably proteins which are directly responsible for its degradation or its synthesis. Figure 2 illustrates the enzymes known to interact directly or indirectly with the sucrose:

- Sucrose phosphate synthase (SPS) (#2), generates sucrose phosphate from fructose-6-P and UDP-glucose.

- Sucrose phosphate phosphatase (#1) immediately cleaves the P from sucrose phosphate to give sucrose. The enzymes work in tandem, but SPS appears to be the rate limiting reaction. Sucrose synthase (#3) is a reversible reaction, either synthesizing sucrose from UDP-glucose and fructose, or cleaving sucrose into UDP-glucose and fructose. In immature tissues, which use a lot of UDP-glucose, the cleavage reaction is favored.

- Sucrose phosphorylase (#5) degrades sucrose in fructose and glucose,

- Sucrose 6-glucosyltransferase (#6), catalyses the reaction that uses the sucrose to build a polymer of polysaccharides.

- Hexokinase (#10) will also synthesize fructose-6-phosphate. This is probably more important than phosphohexoseisomerase (#9). Fructose is also phosphorylated by fructokinase.

- Neutral invertase is active as a monomer, dimer and tetramer, but most of the activity was in the monomer form (Vorster and Botha, 1998). Details of soluble acid invertase from sugarcane are discussed elsewhere (Hussain et al., 2009). Further, excellent summaries on metabolic pathways in sugarcane are given
Variations in the physical and biochemical properties of sugarcane juice before and after

1. Sucrose phosphate phosphatase
2. Sucrose phosphate synthase
3. Sucrose synthase
4. Invertase
5. Sucrose phosphorylase
6. sucrose 6-glucosyltransferase
7. Mannitol dehydrogenase
8. phosphoglucomutase
9. Phosphohexoisomerase
10. hexokinase
11. UDP glucose pyrophosphorylase
12. Glucose6P dehydrogenase

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**Figure 2:** Metabolic pathways in sugarcane

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elsewhere (Rohwer and Botha, 2001; Uys et al., 2007).

### 2.5. Ceramic Membrane

Optimization of sugarcane juice extraction attracts the attention of sugar mills. The sugarcane undergoes many treatments before transformed into juice and then to
crystallized sugar. Although it is impossible to remove all impurities, the aim of is to remove as many as possible especially during clarification step. Sucrose in figure 1 is constituted by two different sugars, also called reducing sugars, glucose and fructose. The degradation of sucrose can be due to acid catalysis, Figure 3 shows how a proton reacts with sucrose to break the link between the two reducing sugar and Figure 4 illustrates the enzyme catalysis. These reactions can change the quality of the juice, because fructose and glucose do not taste as same as sucrose. However, acid catalysis of juice is limited at the early stages because of the ambient temperatures; these reactions are more of a problem downstream where the temperatures are more elevated. Furthermore, reducing sugars can react with proteins under certain conditions and involve in browning or used by microorganisms as a source of amino acids; thus, enzymatic and microbial reactions are most problematic in the juice, particularly microbial. In all these cases the juice will be spoilt.
Table 1: Material characteristics and module details of the membrane system used

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufacturer</td>
<td>Jiangsu Jiuwu HiTech, Nanjing, China</td>
</tr>
<tr>
<td>Membrane type</td>
<td>Tubular</td>
</tr>
<tr>
<td>Membrane material</td>
<td>( \text{ZrO}_2 )</td>
</tr>
<tr>
<td>Membrane support material</td>
<td>( \text{Á-Alumina oxide} )</td>
</tr>
<tr>
<td>Pore size</td>
<td>0.10 ( \mu \text{m} )</td>
</tr>
<tr>
<td>Pure water permeability</td>
<td>533 L/m(^2).h.bar</td>
</tr>
<tr>
<td>Porosity</td>
<td>35%</td>
</tr>
<tr>
<td>Length</td>
<td>500 mm</td>
</tr>
<tr>
<td>Number of channels</td>
<td>19</td>
</tr>
<tr>
<td>Channel diameter</td>
<td>4 mm</td>
</tr>
<tr>
<td>Surface area</td>
<td>0.1193 m(^2)</td>
</tr>
</tbody>
</table>

This study used a ceramic micro-filtration membrane sintered from Alumina or titania, Zirconia oxide under high temperature. The membrane has an asymmetric structure. Ceramic membrane can resist high temperatures, extreme acidity or alkalinity and high operating pressures and it also has a long working life compared to polymeric membranes. Cleaning of ceramic membranes also easier compared to polymeric membranes. Table 1 shows the technical details of the membrane unit used in the experiments and the schematic of the membrane unit is shown in Figure 5. When partially clarified sugarcane juice at 60°C was passed through 0.10 micro meter membrane at a cross flow velocity of 3 m/s, the initial and the average (over a period of 4 hours) fluxes at a TMP of 1 bar were 140.7 and 46.0 L/m\(^2\).h.

3. MATERIALS AND METHODS

Degradation of sucrose content in synthetic sugar solution (15% sucrose) as well as on the raw sugarcane juice was conducted at ambient temperature. Microorganisms and proteins were measured for raw sugarcane juice as well as sugarcane juice after microfiltration (pH: 7.5 and 60°C).

Sample pretreatment: Raw sugarcane juice was extracted from sugarcane by a small milling machine. The raw sugar juice generally contains particles in the range between 0.5 to 2 mm and most of particle carry negative surface charges; this charge must be neutralize to remove the repulsion between the particle in order to coagulate them to separate from the juice. Using of lime allows neutralizing the juice and bringing the pH
to around 7.5. Once the larger particles are settled, the juice was filtrated through a mesh of 250 mm first and then through a 125 mm. The juice was diluted with milli-Q water to obtain a juice with 15% Brix. The juice was heated at 60 °C and placed in the feed tank of the membrane system.

**Physical Measurements:** The quality of the sugarcane juice was evaluated through the measurements of Brix, Pol, color and turbidity.

Brix is a measure of refractometric dry substance (RDS). The juice was filtered through Whatman filter paper and measured Brix by a digital refractometer (Palette PR-101, Atago).

The Pol is a measurement of the total polarized substances in the juice, which is used to represent the sucrose content in the juice. The dry lead method (Bureau of sugar experimentation stations Laboratory for Queensland sugar mills, 1970) was used to measure the Pol. A 2 g of subacetate of lead was mixed with 200 mL of juice and mixed thoroughly. The solution was let several minutes for the precipitate to settle and the supernatant was filtered through Whatman filter paper. A polarimeter (SQF-WXG4, Vanco) calibrated in sugar degree (°Z) was used to measure the Pol reading of the filtered supernatant. The 200 mm length pol tube was filled with the filtered supernatant to determine the Pol reading at 20°C. Pol percent juice was calculated using the following equation:

\[
\text{Pol percent juice} = \frac{\text{Pol reading}}{\text{Pol factor}}
\]
where

\[
\text{Pol factor} = \frac{100 \times \text{apparent density at } 20^\circ\text{C}}{26000}
\]  \hspace{1cm} (2)

\[
\text{Purity of juice (\%)} = \left(\frac{\text{Pol per cent juice/Brix}}{\text{Brix}}\right) \times 100
\]  \hspace{1cm} (3)

\[
\text{Purity rise} = (\text{Purity})_{\text{permeate}} - (\text{Purity})_{\text{feed}}
\]  \hspace{1cm} (4)

The color of the juice was measured according to GS1-7 method (ICUMSA, 1994) where the sample was adjusted to pH 7 by 0.1 N HCl and 0.1 N NaOH and filtered through a 0.45 µm filter in order to measure absorbance at 420 nm using a spectrophotometer (HP - 8453).

\[
\text{Color (IU)} = \frac{10^8 \times A_j}{(b \times \text{RDS} \times \bar{n})}
\]  \hspace{1cm} (5)

Where, \(A_j\) is the absorbance at 420 nm, \(b\) is the cell length (cm), \(\text{RDS}\) is the Refractometric Dry Substances (°Brix) and \(\bar{n}\) is the density of the solution (kg/m³).

The turbidity was measured according to GS 7-21 method (ICUMSA, 1994). Sample was measured at 900 nm using a spectrophotometer (HP - 8453).

\[
\text{Turbidity} = 100 \frac{A_s}{b}
\]  \hspace{1cm} (6)

Where, \(A_s\) is the absorbance at 900 nm and \(b\) is the cell length (cm).

The pH of sugar cane juice solutions was measured by a digital pH meter (AQUA – pH, TPS).

**Chemical Measurements:** Lowry method was used to measure the quantities of proteins (Scopes and Cantor, 1994; Zhang et al., 2003). This method was preferred to the others because it allows the measurement of small quantity of proteins, and an easy treatment of sample allows the constitution of the juice not to interfere with the measurements. The method could analyze proteins in the concentration range of 0.01-1.0 mg/mL. The concentration of proteins in the raw sugarcane juice after stored for 0, 2h, 4h, 7h, 10h, and 24h as well as the permeate from micro-filtration were analyzed.

Many compounds can interfere with Lowry reaction. Samples were filtered with a 0.2 µm filter to remove microorganisms and fibers. The concentration of sucrose in sugarcane juice (15%) was too high to use Lowry method. Therefore the samples were diluted with milli-Q water to obtain the dilutions of 2, 5 and 10. The Lowry reaction involves the formation of a complex between divalent copper ion and peptide bonds to give a monovalent ion. This complex reacts with Folin reagent to produce an unstable product which becomes reduced to molybdenum/tungsten blue. Lysosyme was used to make a standard curve (0.002 to 1mg/mL); absorbance is measured at 650 nm.

**Microbial Measurements:** Population of bacteria, yeast and mould were determined with two media: (i) plate count agar and (ii) malt extract agar (Health Canada, 1998;
Figure 6: Synthetic sugar solution (a) change in Brix (b) change in Pol
2004). A specific kind of bacteria; *lactobacillus* was the focus of the study using De Man Rogosa agar. The samples underwent a serial of dilution until $10^{-9}$ was reached and were inoculated in deep agar. 1mL of each dilution was inoculated in Petri dish (15mL of media) and incubated at 25°C for 3 to 5 days. The experiments were carried out twice.

4.0. RESULTS AND DISCUSSION

4.1. Physical Measurements

**Synthetic sugar solution:** The change in Brix in synthetic sugar solution over a period of 9 hours is given in Figure 6a. The reason for the decrease in Brix could be attributed to transformation of solids into carbon dioxide gas which is when evaporated off, leaving lower amounts of solids. The Brix also changed with pH. At pH 8.5 the Brix was around 15.1%. Change in Brix was similar at pH values 5.5, 6.5 and 7.5, with a value around 14.7%. Thus, lower pH values provoked degradation of sugar. Further, the sucrose concentration in the Figure 6b. decreased quickly (in terms of Pol measurement) at all pH values and after that both production and degradation of sucrose occurred due to forward and backward reactions of acid catalysis. The concentration was not stable during the first four days, but then stabilized around 133 mg/L. Thus, the acid catalysis took place for the whole range of pH tested. However, at acid pH, sucrose degradation is higher due to acid catalysis.

**Raw sugarcane juice:** The variation of pH with time was observed for raw sugarcane juice samples at pH 5.5, 6.5, 7.5 and 8.5. Another sample that was boiled and kept at pH 5.5 was also observed for the change in pH. The pH of the samples did not change during the first 7 hours as Figure 7a shows. After this time the pH began to decrease at different rates, and after 30 hours, the pH decreased around 2 units for all samples. This decrease in pH was due to the development of microorganisms which produced acids. Thus, changes in pH are most likely due to both enzymatic and microbial reactions and could only be attributed very little to forward and backward acid catalyzed sucrose degradation reactions.

During the first 10 hours, Figure 7b shows the changes in turbidity in the raw sugarcane juice was not significant; nevertheless, at the beginning, the result illustrated that the turbidity depended on the pH. The basic pH had more significant effect on turbidity than the acidic pH. The acidic pH could reduce the particles (possibly by dissolving them) in the sugarcane juice and decreased the turbidity. After 10 hours Figure 7b illustrates the turbidity increased at all pH values. This increase in turbidity was due to the growth of microorganisms. This result was correlated well with the change in pH. The decrease in pH was in accord with the exponential growth of microorganisms. Thus, microorganism can deteriorate the sugarcane juice after 7 hours. Further, colloidal and
Solutions to Environmental Challenges through Innovations in Research

(a) pH vs. time (hour)

(b) Turbidity vs. time (hour)
Variations in the physical and biochemical properties of sugarcane juice before and after

(c)

Variations in color (ICUSMA unit) over time (hour) for different pH levels.

(d)

Variations in brix (%) over time (hour) for different pH levels.
pertinacious matters flocculate while heating and increase the turbidity of boiled juice. The Figure 7c illustrates that the color of the raw sugar cane juice did not change until 20 hours. The samples taken at pH 7.5 and 8.5 appeared browner than the samples at pH 5.5 and 6.5; the higher the pH, the browner the color. This was confirmed by the turbidity. But after filtration with watman paper this difference disappeared indicating the color is apparent due to suspended particles. After 20 hours the color increased rapidly at the pH 5.5, but stayed relatively stable at the pH 8.5.

The Brix and Pol of raw sugarcane juice on Figure 7d and 7e, fluctuated between 2.5 and 4.5 hour. At the beginning, when sugarcane was crushed, the sucrose was in the vacuole. After crushing, the sucrose was released into the juice, where the enzymes which used the sucrose were also freed and could catalyze the reactions. Before the sugarcane is cut, there was a strict control of the amount of sucrose in cell, specific enzymes were active when the plant needed energy and they released precise amounts of sucrose from the vacuole which is used in glycolysis, Krebs cycle and respiration. But if the vacuole is broken, then there will be no control and all the enzymes will be freed. In
that case they can use sucrose to produce energy, but if this energy is not utilized, the intermediate compounds will accumulate and react in the reverse direction to synthesis sucrose. In conclusion, some metabolic enzymes are active in the sugarcane juice and they can either consume the sucrose (invertase and the sucrose phosphorylase) or regenerate it (sucrose phosphate phosphorylase and the sucrose synthase). Between 7 and 30 hours the decrease of Brix shows the consumption of sucrose by microorganisms.

The variation of Brix in synthetic sugar solution was very limited (0.2-0.3 % Brix) in two days, whereas in the raw sugarcane juice the variation was around 3-4% Brix. In the raw sugarcane juice there are several compounds which could react with the sucrose: enzymes, bacteria, yeasts, moulds and particles. The reason Brix decreased was mainly due to microbial reactions forming carbon dioxide. Both acid and enzyme catalyses are other possible reasons for this increase in the variation of Brix.

The Pol of raw sugarcane juice was measured between 0 and 10 hours (the juice was too spoiled after this time and the measurement could not be taken thereafter). Pol fluctuated for the samples taken at pH 6.5, 7.5 and 8.5. When Pol decreased there were more fructose and glucose, and when it increased there were more sucrose. At the beginning, the sucrose content decreased, but not due to microbial consumption as their numbers was not sufficient enough to cause such decrease. The change was likely due to the enzyme in the sugarcane juice. At pH 7.5 the decrease was most significant as it was the optimum pH for the neutral invertase activity. The same trend was also observed at the pH 6.5 and 8.5. On the other hand it is also possible that the variations in Pol could be due to the formation of fructose and glucose initially, but then these products break down into further optically active compounds. Furthermore, bacteria can form dextran and mannitol degradation products that have a positive optical rotation, which would have contributed to the variations in pol.

The pH 5.5 is not optimum for the neutral invertase but it is as it optimum for the acid invertase. Nevertheless a small decrease in Pol was observed possibly due the limited action of these two enzymes. The proportion of sucrose increased at around 4 hours at the pH 6.5 and at 7 hours at the pH 7.5 and 8.5. This could be correlated with the growth of microorganisms. The growth would have begun earlier in the sample at pH 6.5 (because this pH represented the optimal condition for microorganisms). When they grow, microorganisms use reducing sugars first as was easier to use them compared to sucrose. The glucose was directly used for the metabolism whereas the sucrose needs to be broken down and as a result the proportion of sucrose should increase. After a while the proportion between sucrose and reducing sugar should become equal as the acidity tends to break the sucrose but the microorganisms consume the glucose and fructose and allow the balance to be maintained.
Variations in the physical and biochemical properties of sugarcane juice before and after 

Lime treated and membrane filtered sugarcane juice: After filtration sugarcane juice (permeate) was very clear and Figures 8a, 8b and 8c show the color was reduced by the treatment, but the Brix and Pol were not affected much. The color was more significant in the feed solution (lime treated raw sugarcane juice) compared to the permeate. The browning was due to the Maillard reaction of particles. In fact the dehydration step between reducing sugars and proteins illustrated in Figure 1 is facilitated by the heating, thus Maillard reaction occurs easier in the feed than in the raw sugarcane juice. However, micro-filtration was efficient in removing the color and the permeate appeared less brown than the raw sugarcane juice.

The change in Brix was very similar for the samples of feed ad permeate taken every hour of filtration. Pretreatment and microfiltration did not have an effect on the change in Brix; sucrose and reducing sugar were stable. The membrane and the pretreatment reduces the population of microorganisms significantly, thus nothing can degrade these molecules. Even if the enzyme are also active they do not degrade the carbohydrate, they just transform them into other compounds. Nevertheless, between the feed and permeate a difference of around 1% Brix (more in the feed) was observed. That means the membrane has rejected some sucrose. Several compounds such as fiber, starch, lipids,
could be accumulated at the surface of the membrane and prevent the complete passage of sucrose.

Pol was very similar in the feed and permeate but not in the raw sugarcane juice which is significantly lower compared to the feed and the permeate. The pretreatment probably stopped or slowed the enzyme responsible for the degradation and the formation of sucrose. The pretreatment and the filtration removed most of the impurities which were responsible for the color and they could also have an inhibitory effect on the metabolic enzyme.

4.2. Chemical Measurements

**Raw sugarcane juice:** The Figure 9 shows the amount of proteins in the raw sugarcane juice, it was increased until 7 hours and decreased afterword. From the physical measurements such as turbidity showed the growth of microorganism in the raw sugarcane juice to commence around 7 hours. To multiply, the microorganisms needed amino acid sources where they could find proteins. Firstly, they could consume the protein, including the enzymes responsible for the sucrose metabolism, implying that after 7 hour no
correlation could be made between sucrose and enzymes. After 20 hours, the stress due to pH and excessive microbial growth could entail the decay of microorganisms, which could be shown by an increasing and then a stabilization of the protein concentration.

**Permeate from microfiltration:** Two permeate samples were collected between 30 minutes interval. These samples were correlated with the sample in the raw sugarcane juice after 4 hours and 7 hours and the results are given in Table 2. The first sample had a high quantity of proteins but not the second sample. An accumulation of impurities on the membrane must have retained part of proteins on the membrane surface. The raw sugarcane juice contains 0.5% of protein in the dry solids and most of the protein in the raw sugarcane juice is coagulated due to liming and heat and removed by clarifier. After membrane filtration, the protein content in the dry solids comes down to 0.11%.

### 4.3. Microbial Measurements

**Raw sugarcane juice:** The enumeration was commenced 5 hour after the crushing of sugarcane as 5 hours are required to complete the pretreatment. The raw sugarcane juice had many microorganisms; the enumeration showed around $7.9 \times 10^7$ yeast and mould, $3.5 \times 10^7$ bacteria and $4.5 \times 10^4$ lactobacilli per mL of sample. The contamination could have happened at the following steps:

- Harvesting, when the sugarcane was cut, the microorganism on the soil could have easily infected the extremity of the plant.
- Storage, during this step, the quantities in bacteria, yeast and mould increased significantly, each day.
- Crushing, when the sugarcane underwent this treatment the microorganisms in apparatus, in the ground and in containers could contaminate the juice.

**Permeate:** The permeate contained around 120 yeast and mould, 121 bacteria and less than 10 lactobacilli per mL of sample. The log removal of yeast and mold, bacteria and lactobacilli by 0.10 µm ceramic membrane is 5.82, 5.46 and 3.45 respectively. When

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**Table 2: Comparison of protein concentration in the raw sugarcane juice and the permeate of microfiltration**

<table>
<thead>
<tr>
<th></th>
<th>Protein concentration (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw sugarcane juice after 4 hours</td>
<td>1.74</td>
</tr>
<tr>
<td>Raw sugarcane juice after 7 hours</td>
<td>1.90</td>
</tr>
<tr>
<td>Permeate of microfiltration (initial sample)</td>
<td>2.40</td>
</tr>
<tr>
<td>Permeate of microfiltration (after 30 minutes)</td>
<td>1.47</td>
</tr>
</tbody>
</table>
comparing the sizes of the microorganisms with the pore size of the membrane used in this study, the log removal values are acceptable. The microorganisms could not cross the membrane because the membrane pore size was too small, but they were definitely developed in permeate pipe of the membrane system and infected the juice after filtration. For example, size of yeast size can vary greatly depending on the species, typically measuring 3 to 4 \( \mu \text{m} \) in diameter, although some yeasts can reach over 40 \( \mu \text{m} \). The size of bacterial cells including lactobacilli is more than 1 \( \mu \text{m} \). Thus, the membrane used in this study with a pore size of 0.1 \( \mu \text{m} \) should be able to retain all the microorganisms mentioned above. However, their presence in the permeate indicates possible contamination of permeate pipe with those microorganisms. This problem is not very significant in sugar mills as next the step of filtration would be the evaporation and crystallization where the juice will be heated above 100°C. Microorganisms can not survive at this temperature as the heating is too intense and there is very little amount of water present.

5. CONCLUSIONS

The studies on the raw sugarcane juice and synthetic sugar solution allowed understanding the reactions that occur in the juice. When the sugarcane is harvested the microorganisms can develop in the cane. After crushing they enter the sugarcane juice which is a medium more than compatible for their growth and development and therefore they can grow faster than before and will become a problem after 7 hours. Furthermore, the crushing releases many compounds such as sucrose, lipid, fiber and protein from the sugarcane. The sugarcane cells are constituted of several organelles such as nucleus, mitochondria, chloroplasts, cellular membrane, lysosome and vacuole. Molecules such as sucrose are kept inside of them and their release is strictly controlled by the cells. When the sugarcane is crushed these molecules are free in the juice, and particularly the sucrose as it is the major component, can react in several chemical and enzymatic reactions. In the juice, reactions depend only on the equilibrium between the components. Therefore the sucrose is formed when it is less in the juice and when it is in excess quantities, it degrades. This is similar to glucose, fructose and other intermediate molecules. Fluctuation in sugar content can be due to this phenomenon. After 7 hours the microorganisms become very significant and deteriorate the juice by consuming glucose, fructose (then sucrose) and proteins. While it is true that the soluble enzymes will be in the juice, the concentrations of intermediates, the pH, and the temperature, will all influence how active any of them is. The important ones in the sucrose factory will be invertases. However, it was not the scope of this study to evaluate the factors mentioned above and those factors should be considered in further studies.

The micro-filtration is really efficient in reducing the browning of juice. Proteins in the juice are too small to be rejected and they can pass through the membrane. Nevertheless
the accumulation of impurities on the membrane can increase the rejection of protein during filtration. Proteins are bio-molecules and they have specific characteristics such as hydrophobic, hydrophilic and charged positive or negative. These specific properties allow them to react, to be produced, to be structured and are responsible of bonds within the molecule or between the enzyme and other compounds (in hydrophobic chromatography the hydrophobic quality is used to trap the hydrophobic proteins. Thus, they can easily build bonds between themselves and the impurities on the membrane such microorganisms, fiber and lipids.

When microorganisms are concerned, the lime pretreatment is sufficient to remove the most of them but they are always found in micro-filtration permeate since the experimental conditions are not sterile and could let the microorganisms infect the permeate. To be efficient it is necessary to keep sterile conditions, nevertheless removing a large portion of microorganism can extend the shelf life of the sugarcane juice.

ACKNOWLEDGMENTS

Sugar Research and Development Corporation funded a project entitled “JCU029: Evaluation of membrane technology for clarification of sugarcane juice (2007-2009)” to carry out this work. Messrs. Curt Arrowsmith, Stuart Petersen and John Ellis of the School of Engineering, James Cook University are acknowledged for their support in installing and operating the membrane unit. We sincerely thank the anonymous reviewers whose comments have made this chapter more useful.

REFERENCES


