OBTAINING OF ETHANOL AS AUTOMOTIVE FUEL BY SOLID WASTES RECYCLING THROUGH S. CEREVISIAE OR Z. MOBILIS FERMENTATION

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Abstract: Solid wastes are still problems of every city in Indonesia. In general, solid wastes are only transported from the Temporary Disposal Site (TDS) with dump trucks to the Final Disposal Site (FDS). Water, soil and air pollutions happen, make local people reject the presence of FDS. Making ethanol from solid wastes undertaken from TDS Gebang Putih, Surabaya city was expected to solve the problem of city solid wastes. It is therefore necessary to study the ethanol extraction from the city solid wastes to see their potential as a renewable energy.

Solid wastes from the polling stations (TDS) were then added to water with a ratio of 75%: 25% and 50%: 50% before mashed with a blender and heated 100 ° C for the hydrolysis process, to kill pathogenic bacteria and waste extraction. Furthermore, solid waste mixtures were fermented with Saccharomyces cerevisiae and Zymomonas mobilis for 3, 5 and 7 days.

In this study, highest ethanol production by Saccharomyces cerevisiae was at 8.05%. The concentration of the best waste slurry in producing the highest amount of ethanol is similar, whether solid wastes to water ratio of 75%:25% or 50%:50%. While the highest ethanol production by Zymomonas mobilis was 9.51% at solid wastes to water ratio of 50%:50%. While the concentration of solid wastes to water of 75%: 25% only yielded 9.43%. The fermentation time of the most highly produced ethanol for both, yeast and bacteria were 7 days.

Key words: solid wastes, fermentation, distillation, ethanol, yeast, Zymomonas mobilis.

1. INTRODUCTION

Waste generation rate in Surabaya as one of the big city in Indonesia is high. A large number of wastes generated in the city can not be separated from the high birth rate and rapid urbanization in large cities. This condition is further exacerbated by the lack of public awareness on proper waste handling.

It is important therefore, to take concrete steps, to convert the solid wastes into something useful due to the inadequate solid waste handling. Various studies and attempts have been made to find ways to resolve the issue (Gunasekaran, P. and Raj, K. C. 1999). One way that could be used to overcome this problem is converting solid wastes into ethanol for various purposes. Sugars contained after hydrolysis of the solid wastes are converted into ethanol through an anaerobic fermentation process assisted by microorganisms (Wilkie et al, 2000).

Yeast and bacteria are among others the microorganisms that can help the fermentation process of ethanol. Yeast that can help this process is *Saccharomyces cerevisiae*, whereas one of the bacteria that may play a role in this process is *Zymomonas mobilis* (Themelis et al, 2002) (Najafpour, G. D., Lim, J. K. 2002).

Saccharomyces cerevisiae fermentation to produced ethanol has several drawbacks, among others, they can only ferment the carbohydrates glucose, sucrose or fructose and can not stand with the high concentration of ethanol, while the *Zymomonas mobilis* bacteria, although naturally only to ferment carbohydrates of the same type, but these bacteria can survive in high ethanol concentration, which mean that they can produce more ethanol (Yamashita, 2008). The advantage of using yeast is no media culture is required..

Solid wastes from Gebang Putih Village as part of the Surabaya and the surrounding area are temporally disposed of at the Temporary Disposal Site (TDS) Gebang Putih. Type of solid wastes contained therein are household wastes, and trash food stalls. Content of organic wastes found in the solid wastes at the polling stations can be used as raw materials for ethanol production through an anaerobic fermentation process.

2. MATERIAL AND METHODS

2.1 Preparation of research

Solid wastes were retrieved from the TDS Gebang Putih Surabaya. Solid wastes were taken at random from four different points. The tools needed for sampling were a plastic bag, gloves and a shovel. Solid wastes were inserted into a plastic bag.

After being collected, solid wastes were then prepared for sorting by type, namely: easy rotting solid wastes, paper wastes, food wastes and other wastes. Then each type of solid wastes was weighed and ready for observation.

For smoothing the size of solid wastes, an electric blender was used. For the hydrolysis process, an

aluminum pan and electric stove were used. For the fermentation process, 250 ml erlenmeyer tubes were used. While for the distillation process, a laboratory scale distillation equipment was used.

2.2 Grinding of solid wastes

Solid wastes prepared based on the type were then weighed. Easy to decompose organic solid waste, were taken as much as 1000 mg. The solid wastes were then further subdivided into two parts, namely 400 mg and 600 mg solid wastes. The 400 mg solid wastes were mixed with 400 ml of clean water. Mixing was done by putting the 400 mg solid wastes and the 400 ml of clean water into the blender and stirred. Ratio between the solid wastes to the water by weight was 50%: 50%.

Another mixture was the 600 mg of solid wastes and the 200 ml of clean water. Mixing was also using the similar blender. Solid wastes to water by weight ratio was 75%: 25%.

2.3 Heating of solid wastes

After mixing and crushing of solid wastes and water into a solid waste slurry, the solid waste slurry was then heated to the boiling point (100^{o} C), as shown in Figure 1. This was aimed to eliminate the pathogenic bacteria contained in the solid waste slurry. In addition, heating is intended to simplify the structure of sugar in the solid waste slurry so that it will ease the process of ethanol fermentation by *Zymomonas mobilis* bacteria and *Saccharomyces cerevisiae* yeast.

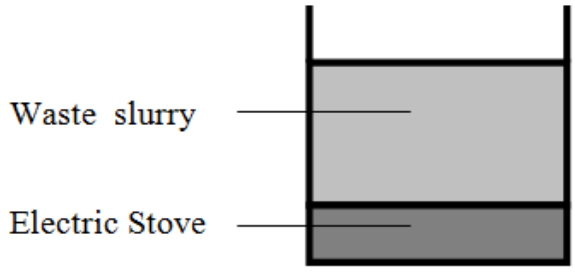

Fig. 1. Hydrolyisis Equipment

After the heating process was completed, solid waste slurries with solid wastes to water ratio of 75%: 25% and 50%: 50%, for each fermentation process were equally poured into six 250 ml erlenmeyer, each filled with 120 ml of solid waste slurry.

2.4 Media culture preparation

Powdered NBA media prepared in a beaker glass was diluted in 100 ml distilled water. It was then heated up to 100^{o}C and inserted into three 7 ml reaction tubes. The reaction tubes were then closed with cotton plug. These tubes were then put into a beaker glass and covered with wax paper and tied up with a rope.

After the media for the bacteria growth had actually been closed, the beaker glass was then put into an autoclave. This was intended to sterilize the media. After sterilization process, the media were taken out. The media were then tilted in an approximately 25-39^{o}C to dry without removing the cotton plug.

The media in the reaction tubes were ready for use. Ose needle was heated to red. The cotton plug in the reaction tube was opened. The reaction tube mouths were passed on fire as much as 2-3 times. Ose needle that had been sterilized was inserted into the mouth of a reaction tube to take a bacterial culture. Cotton plug of the reaction tube containing bacterial culture was then pluged back. The Ose needle containing bacterial culture was inserted into a reaction tube with the NBA media.

2.5 Sugar content analyses

Sugar content analyses were undertaken before and after the fermentation process. Sugar content analyses were done in BPKI laboratory Surabaya.

2.6 Fermentation processes

The fermentation processes were using *Zymomonas mobilis* and *Saccharomyces cerevisiae*. The samples were inoculated with bacterial cultures as much as five times, or samples were fermented by *Saccharomyces cerevisiae* as much as 5 grams yeast, put into 250 ml erlenmeyer and closed with insulation paper or plastic and tied with a rope. Having ascertained that there was no air coming into the erlenmeyer, the samples were then fermented at room temperature (25-30^{o}C) (Zhao and Xia, 2010). Samples were fermented by yeast and bacteria for 3 days, 5 days and 7 days (Murniasih, 2008).

The total number of samples fermented by *Zymomonas mobilis* were 6 units consisting of: (a) sample fermented for 3 days with solid wastes to water ratio of 50%: 50%, (b) sample fermented for 3 days with solid wastes to water ratio of 75%: 25% , (c) sample fermented for 5 days with solid wastes to water ratio of 50%: 50%, (d) sample fermented for 5 days with solid wastes to water ratio of 75%: 25%, (e) sample fermented for 7 days with solid wastes to water ratio of 50%: 50%, and (f) sample fermented for 7 days with solid wastes to water ratio of 75%: 25%.

The total number of samples fermented by *Saccharomyces cerevisiae* were also 6 units consisting of: (a) sample fermented for 3 days with solid wastes to water ratio of 50%: 50%, (b) sample fermented for 3 days with solid wastes to water ratio of 75%: 25%, (c) sample fermented for 5 days with solid wastes to water ratio of 50%: 50%, (d) sample fermented for 5 days with solid wastes to water ratio of 75%: 25%, (e) sample fermented for 7 days with solid wastes to water ratio of 50%: 50%, and (f) sample fermented for 7 days with solid wastes to water ratio of 75%: 25%, as shown in Figure 2.

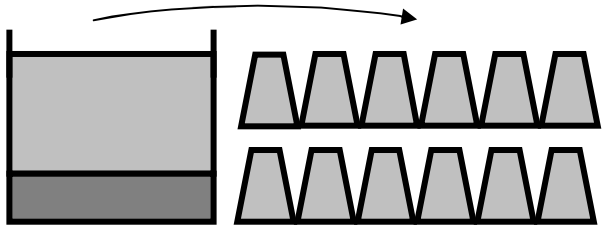

Fig. 2. Hydrolysis and fermentation equipments

Image description: (a) Heating and stirring pot is made of aluminum, serves to heat the waste slurries, (b) Heater used was an electric stove, (c) Waste slurry for fermentation used 250 ml erlenmeyer, and (d) Fermentation was carried out at temperature of 25°C .

2.7 Test on initial ethanol content

Initial test on ethanol content after fermentation was conducted in BPKI Laboratory Surabaya and at the Laboratory of Environmental Engineering ITS using a pycnometer.

2.8 Distillation

After all samples were completely fermented and analyzed for ethanol contents, the samples were distilled at the Laboratory of Environmental Engineering ITS Surabaya.

Samples to be distilled were put into 250 ml erlenmeyers and heated to a temperature of 80^{o}C. In order to the ethanol steam turns into liquid, the condenser pipe was flowed by water from the faucet. The distilled ethanol are accommodated in 250 ml erlenmeyer (Olujić et al, 2009).

3. RESULTS AND DISCUSSION

3.1 Inoculation of *Zymomonas mobilis* bacteria

Zymomonas mobilis bacteria was inoculated on the NBA media. This bacteria was inoculated from pure cultures of *Zymomonas mobilis* in the Laboratory of Chemical Engineering ITS Surabaya. After the inoculation process is completed, reaction tubes containing media were sealed with cotton. Then the media was kept at room temperature (± 25ºC). After five days the bacteria can be visually observed. Media surface turned white. Colors indicate the culture of the bacteria.

Zymomonas mobilis bacteria that had been cultured in the medium were then observed with a microscope. This is to indicate whether the physical form of the bacteria has the same physical form obtained from the literature, as shown in Figure 3.

3.2 Solid waste preparation

For this study solid wastes were taken randomly at the TDS Gebang Putih Surabaya. Collection was done during the day before the garbage truck comes to haul trash to the landfill. Solid waste collection of approximately 15 kg of solid waste was done by using hands that had been protected with plastic gloves.

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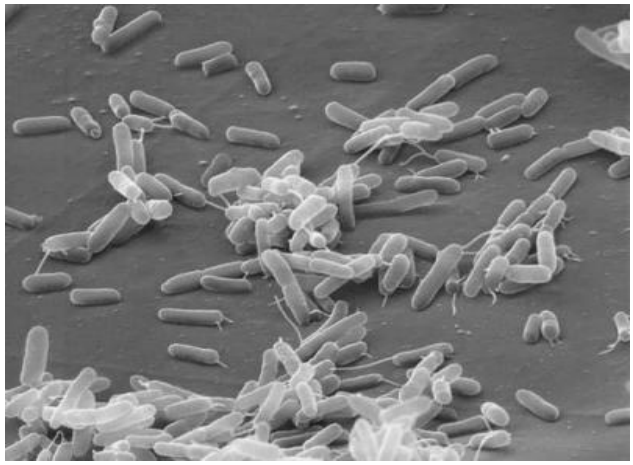

Fig. 3. Optical enlargement (zoom) of *Zymomonas mobilis* ZM4 (NRRL B-14234) by an elektron microscope

3.3 Solid waste identification

After collection, solid wastes were separated based on their composition and calculated for each species by using the formula:

$$(A / 10 \text{ kg}) \times 100\%$$

The percentage of each waste category was: organic wastes 42.66%, paper wastes 24.00%, plastic wastes 21.33%, trash timber 7.33%, trash etc 4.66%.

3.4 Solid waste grinding

Solid wastes of organic materials collected were then separately weighed to be used as raw materials. In this research, organic wastes were mashed into pulp with a comparison of: (a) 600 mg solid wastes and 200 ml of water as a solid waste to water ratio of 75%: 25%, (b) 400 mg solid wastes and 400 ml of water as a solid waste to water ratio of (50%: 50%).

The solid wastes were then mashed with a blender until completely soft and smooth. After the smoothing process was complete, solid wastes to water ratio of 75%: 25% seemed to be more brownish (more concentrated) and stinking when compared to pulp which had a solid wastes to water ratio of 50%: 50%. Furthermore, solid waste slurries were placed in an aluminum pan for the hydrolysis process.

3.5 Heating of solid waste slurries

The solid waste slurries were heated in an aluminum pan up to 100^{o}C. After heating process (hydrolysis) over the samples, the sample volumes were automathically reduced to 10-20 ml. These were

because the water contained in the solid waste slurries was evaporated. These resulted in more concentrated solid waste slurries than the previous slurries.

3.6 Analysis of sugar contents before fermentation

After going through the process of heating, samples with solid wastes to water ratio of 75%: 25% and 50% : 50% were tested for sugar content. The sugar contents of both ratios are as follows: (a) solid wastes to water ratio of 75%: 25% = 10.82% sugar content, (b) solid wastes to water ratio of 50%: 50% = 9.96% sugar content.

Based on the data obtained, it is known that the highest sugar content was found in samples that have solid wastes to water ratio of 75%: 25%. This is because the substrate content in these samples has more than the samples that have solid wastes to water ratio of 50%: 50%, so that the sugar contents in the samples were also more.

3.7 Ethanol fermentation processes

3.7.1 Fermentation by yeast

Three samples of solid waste slurries with solid wastes to water ratio of 75%: 25% and three samples of solid waste slurries with solid wastes to water ratio of 50%: 50% were fermented by yeast. Samples fermented for three days, had a rotting garbage smell, no change of color and volume. Samples fermented for five days, still had a rotting garbage smell, no change of color and volume. Samples fermented for seven days, still had the smell of rotting garbage but started to smell ethanol. Samples were still with no change in color and volume.

3.7.2 Fermentation by *Zymomonas mobilis* bacteria

This process was similar to the yeast fermentation process. It differ only by the use of *Zymomonas mobilis* bacteria that had been cultured on media stored in an incubator for fermentation process. Fermentation was performed for 3, 5 and 7 days. During this time the samples were stored at room temperature (± 25^{o}C). Samples fermented for 3 days was still stink, no change in color and volume. Samples fermented for 5 days smell rotten garbage, no change in color and volume. Samples fermented for 7 days start to had the smell of ethanol. But the volume and color changes are not visible.

3.8 Filtering of solid waste slurries

After the fermentation process was complete, the samples were subsequently filtered using a cloth. This was part of steps to purify the sample. The volumes of solid waste slurries after filtered are shown in Table 1 and 2.

Table 1. The volume of each sample observed from the yeast fermentation after being filtered

Solid wastes to water ratio	Waste volume (ml)		
	3 days	5 days	7 days
50%: 50%	63	62	78
75%: 25%	56	55	58

Table 2. The volume of each sample observed from *Zymomonas mobilis* fermentation after being filtered

Solid wastes to water ratio	Waste volume (ml)		
	3 days	5 days	7 days
50%: 50%	40	61	58
75%: 25%	54	58	55

The samples were filtered and separated for later ethanol content analyses. Screening process resulted in a more dilute sample because only a few solids left in the solution after filtration. This will affect the ethanol content in the sample.

3.9 Analyses of sugar contents after fermentation

3.9.1 Analyses of sugar content after yeast fermentation.

Each sample was filtered prior to sugar content test. Results of the sugar content tests for each sample after yeast fermentation are shown in Table 3.

Table 3. Sugar content of each sample after yeast fermentation

Solid wastes to water ratio	Sugar content (%)		
	3 days	5 days	7 days
50%: 50%	4.93	3.62	2:01
75%: 25%	5:38	2.74	1.92

From the data obtained, it can be seen that sugar levels were significantly declining. This was due to the fermentation process of sugar contained in the samples into ethanol.

Samples with solid wastes to water ratio of 50%: 50%, fermented for three, five and seven days, declined in total sugar content by 5, 03%, 6:34% and 7.95% respectively.

Samples with solid wastes to water ratio of 75%: 25%, fermented for three, five and seven days, declined in total sugar content by 5.44%, 8.08%, and 8.90% respectively.

3.9.2 Analysis of sugar content after fermentation by *Zymomonas mobilis*

Analysis of sugar content after fermentation by *Zymomonas mobilis* are shown in Table 4.

Table 4. Sugar content after fermentation by *Zymomonas mobilis*

Solid wastes to water ratio	Sugar content (%)		
	3 days	5 days	7 days
50%: 50%	4.11	2.86	1.03
75%: 25%	3.88	2.60	1.02

Just as the samples fermented by yeast, in these samples also experienced decreases in sugar contents. Among the fermentation time of 3, 5 and 7 days, seven days showed the lowest sugar content.

Samples with solid wastes to water ratio of 50%: 50%, fermented by *Zymomonas mobilis* for three, five and seven days, declined in total sugar contents each of 5.85%, 7.10% and 8.93% respectively. Samples with solid wastes to water ratio of 75%: 25%, fermented by *Zymomonas mobilis* for three, five and seven days, declined in total sugar contents amounted to 6.94%, 8.08%, and 9.80% respectively.

From the data obtained, it was not known on how long the sugar levels will undergo complete depletion. This was because the longest fermentation time in this study was seven days.

3.10 Analysis of ethanol levels after fermentation

3.10.1 Analysis of ethanol content after fermentation by yeast

Results of laboratory tests on the ethanol content of each sample are shown in Table 5.

Table 5. Levels of ethanol after fermentation by yeast in each sample

Solid wastes to water ratio	Ethanol content (%)		
	3 days	5 days	7 days
50%: 50%	5.05	6.30	8.05
75%: 25%	5.64	7.44	8.05

From the data obtained, the highest ethanol contents were obtained from the day seven. Increased level of ethanol occurs when the levels of sugar in the substrate turned into ethanol through anaerobic fermentation.

Samples with solid wastes to water ratio of 50%: 50%, fermented for three, five and seven days, increased in ethanol content of 5,05%, 6.30% and 8.05% respectively. Samples with solid wastes to water ratio of 75%: 25%, fermented for three, five and seven days increased in ethanol content of 5.64%, 7.44%, and 8.05% respectively.

3.10.2 Analysis of ethanol content after fermentation by *Zymomonas mobilis*

Ethanol content test results obtained from the BPKI Laboratory Surabaya for each sample through fermentation by *Zymomonas mobilis* are shown in Table 6.

Table 6. Levels of ethanol of each sample after fermentation by Zymomonas mobilis

Solid wastes to water ratio	Ethanol content (%)		
	3 days	5 days	7 days
50%: 50%	5.12	7.13	9.51
75%: 25%	6.30	7.21	9.43

Increased level of ethanol from three, five and seven day fermentation periods also experienced by the samples fermented by *Zymomonas mobilis*. These increases occurred because the sugar contained in the samples turned into ethanol through fermentation processes. The optimum fermentation time was unknown because until the day seven, glucose levels were still declining. It would then be calculated and compared: the increased of ethanol contents and the decreased of sugar levels in the samples.

Samples with solid wastes to water ratio of 50%: 50%, fermented for three, five and seven days, increased the levels of ethanol, each for 5,12%, 7.13% and 9.51% respectively.

Samples with solid wastes to water ratio of 75%: 25%, fermented for three, five and seven days, increased the levels of ethanol, each for 6:30%, 7.21%, and 9.43% respectively.

3.11 Distillation process

The samples were heated at distillation temperature of not exceeding 80° C. This was aimed to avoid any other substances of being evaporated together with the ethanol. Ethanol that evaporates was then cooled by flowing water and captured liquid ethanol in a 250 ml erlenmeyer.

After distillation, colors of the samples become more clear. In addition, if the sample is inhaled it will smell ethanol. Ethanol that had been distilled, were put in a bottle prepared for measuring the volume and ethanol content. Observations on the final volume of the sample after distillation (yeast fermentation and *Zymomonas mobilis*) are shown in Figure 4.

After distillation, the sample volumes decreased drastically. These were due largely to the small distilled ethanol substances.

Substances that were not ethanol was left in the erlenmeyer as a residue of a fermentation process.

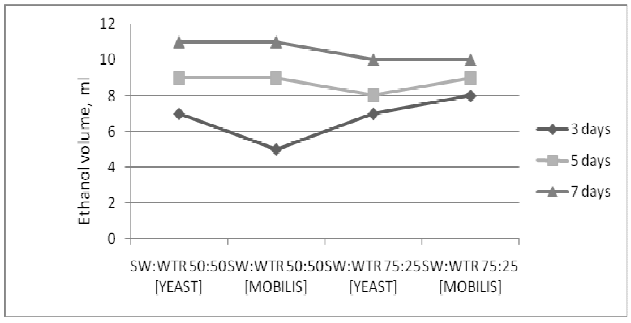

Fig. 4. Final volume of the samples after distillation (yeast and *Z. mobilis* fermentations)

3.12 Test on ethanol contents after distillation

After going through the stages of distillation, the sample was then tested by burning. If the resulting flame is blue, then the sample actually contains ethanol.

3.13 Test on ethanol contents of samples after distillation (yeast fermentation)

Results of tests on ethanol contents of each sample after distillation (yeast and *Z. mobilis* fermentation) are shown in Figure 5.
Based on the data obtained, the ethanol contents of the fermented samples for seven days were the highest followed by the fermented samples for five and three days.

3.14 Ethanol contents of samples after *Zymomonas mobilis* fermentation and distillation

Content of ethanol from fermentation by *Zymomonas mobilis* after distillation is shown in Figure 5.

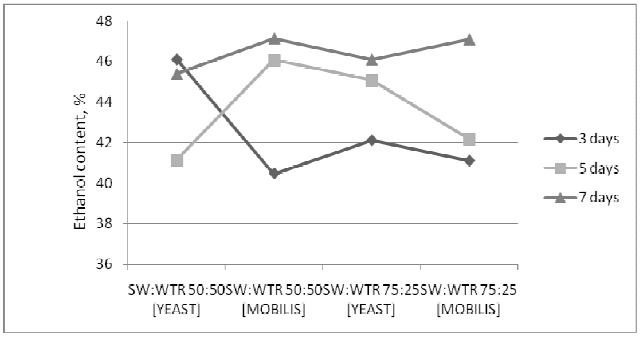

Fig. 5. Content of ethanols after distillation by yeast and *Z. mobilis* fermentation

4. CONCLUSIONS

Zymomonas mobilis is more effective for the manufacture of ethanol than yeast in the fermentation of ethanol from solid wastes. Ethanol fermentation by yeast for seven days with solid wastes to water ratio of 75%:25% and 50%: 50% yield as much as 8.05% ethanol. While the ethanol fermentation by *Zymomonas mobilis* with similar fermentation time and solid wastes to water ratio of 50%:50% yield 9.51% ethanol and solid wastes to water ratio of 75%:25% yield 9.43% ethanol.
The higher the solid wastes to water ratio, the higher the ethanol contents after fermentation and distillation processes. In the solid waste slurry fermented by yeast for seven days, the solid wastes to water ratio of 50%: 50% yield 45.40% ethanol after distillation. While, the solid wastes to water ratio of 75%: 25% yield 46.11% ethanol after distillation.
From the 3, 5 and 7 day fermentation time and first step distillation data, it is concluded that 7 day fermentation followed by distillation is the most effective time to produce ethanol (47.14%), followed by 5 days (46.07%) and the 3 days (40.47%) through *Zymomonas mobilis* fermentation and distillation.
With one more stage of distillation, the ethanol content could be increased to 95% which can be used for motor vehicle fuels, through a mixture of 10, 20, 50 or 85% ethanol and gasoline. Flue gas will be better because it only contains CO_2 and H_2O, thereby reducing the impact of global warming. Municipal solid wastes will be more utilized.

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