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Effectiveness of Powder and Solid Ragi Starter on Production of Bioethanol from Rice Straw Hydrolyzate

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Abstract

Rice straw is one of the most abundant forms of agricultural wastes in Indonesia. Rice straw contains polysaccharide in the form of cellulose and hemicellulose, which can be used as raw materials in the production of bioethanol. This study aims to examine the effectiveness of bioethanol production from rice straw's hydrolyzate. Research was carried out by fermenting the sample using two types of starters (Baker's yeast, known as powder starter (F); and Tapai's starter, known as solid starter (S) with a control of pure yeast, *Saccharomyces cerevisiae*. Glucose levels were measured using a glucometer, and bioethanol levels were analyzed using high-performance liquid chromatography (HPLC). In this study, split plot design was used as a data analyzer with two treatment factors: starter's inoculum (R) and time of fermentation (T). This study shows that there is no significant difference between the starters' levels of bioethanol production. However, S's treatment produced higher levels (5,1 g/L) of bioethanol compared to F's (4,8 g/L); the rate of bioethanol production in S's treatment is also higher (1,3 g/L.h) than that in F's (1,2 g/L.h). This study concludes that Tapai's starter is more effective in producing bioethanol from rice straw's hydrolyzate.

Abstrak

Efektivitas Produksi Bioetanol dari Hirolisat Jerami Padi Menggunakan Ragi Bubuk dan Ragi Padat. Jerami padi merupakan salah satu limbah pertanian yang sangat melimpah di Indonesia. Jerami padi mengandung polisakarida dalam bentuk selulosa dan hemiselulosa, yang dapat dimanfaatkan sebagai bahan baku dalam produksi bioetanol. Penelitian bertujuan melihat efektivitas produksi bioetanol dari sampel hidrolisat jerami padi dengan menggunakan ragi roti (ragi bubuk – F) dan ragi tapai (ragi padat – S). Penelitian dilakukan dengan memfermentasikan sampel menggunakan kedua jenis ragi tersebut, dan isolat murni khamir *Saccharomyces cerevisiae* sebagai kontrol. Kadar glukosa diukur menggunakan *glucometer* dan kadar bioetanol dianalisis menggunakan *high-performance liquid chromatography* (HPLC). Penelitian menggunakan *split plot design* dengan dua faktor perlakuan; pemberian ragi (R) dan waktu fermentasi (T). Hasil penelitian menunjukkan bahwa kedua jenis ragi pada produksi kadar bioetanol dari sampel memberikan pengaruh yang tidak berbeda nyata. Namun perlakuan S menghasilkan kadar bioetanol yang lebih tinggi (5.1 g/L) dibandingkan dengan perlakuan F (4.8 g/L); laju produksi bioetanol pada perlakuan S juga lebih tinggi (1.3 g/L.j) dibandingkan dengan laju produksi bioetanol pada perlakuan F (1.2 g/L.j). Kesimpulan dari penelitian adalah penggunaan ragi Tapai lebih efektif dalam memproduksi bioetanol dari hidrolisat jerami padi.

Keywords: agricultural waste, bioethanol, fermentation, starter, rice straw

1. Introduction

Nowadays, bioethanol is produced from first generation feedstock such as sap sugar/sucrose or starchy substances. This causes fierce competition among the producers of food sources and falls under suspicion as one of the causes of the rising value of foods [1]. In fact, bioethanol can also be produced from second generation feedstock: organic waste, wood and forestry waste, plantation waste, and agricultural waste [2]. Bioethanol can be mixed into gasoline and used as an alternative energy source for fossil fuels. Therefore, many researches try to obtain a new potential material for producing bioethanol and the effective method, instead.

Rice straw is a form of agricultural waste in Indonesia that, due to its consistent annual increase [3], has the highest potential bioethanol food-stock [4]. Rice straw possesses many benefits, among them use as livestock and organic fertilizer. Despite these uses, most rice straw is dumped or even burned, which causes water (odour) [5] and air (producing methane) pollution [6].

The major components of rice straw are lignin, cellulose and hemicelluloses [7], known as lignocellulosic material. As carbohydrate components, cellulose and hemicelluloses can be degradated by microorganisms (generally with the *Saccharomyces cerevisiae*), and therefore produce bioethanol [8].

Rice straw bioconversion into bioethanol is conducted through several processes, beginning with the pretreatment, hydrolysis, and fermentation. Pretreatment can be conducted physically or mechanically, either by quickly pressing and steaming (steam exploding), or conducted chemically by soaking with lime or chemicals that can break open lignin protection for a specific period. Once the protective lignin is 'soft', then the rice straw is ready for hydrolyzation [9].

Hydrolysis can be conducted using two methods, acid hydrolysis or enzymatic hydrolysis. Acidic hydrolysis is conducted by cooking the straw, which is diluted in sulphuric acid under high temperature and high pressure conditions [9]. Enzymatic hydrolysis uses hemicellulose enzymes, which have the ability to break down cellulose into glucose [10]. Use of enzymes is more efficient in hydrolyzing the cellulose, and can be combined with the fermentation process known as simultaneous saccharification and Fermentation (SSF). There is also a Separate Hydrolysis and Fermentation (SHF) method in which the enzymatic saccharification is conducted before fermentation. Hydrolysis during SHF is completed in two stages. In the first stage, most of the hemicelluloses and a small amount of cellulose will split into its constituent sugars, while the second phase aims to break down the remaining unhydrolyzed cellulose. The second hydrolysis phase is expected to obtain a large quantity of sugar [7].

Obtained hydrolyzate is fermented with an anaerobic fermentation that uses yeast *Saccharomyces cerevisiae* to convert glucose into ethanol. One molecule of glucose produces 2 molecules of ethanol and 2 molecules of CO_2. Theoretically, one kilogram of glucose will yield 0.51 kilograms of ethanol and 0.49 kilograms of CO_2 [11].

S. cerevisiae yeast is contained in F (powder starter type) and S (solid starter type). Both F and S can be found on the traditional market, and so are easily obtainable by the public [12]. Although both of the commercial starters contain *S. cerevisiae*, their relative effectiveness in fermenting rice straw hydrolyzate to produce bioethanol has never been compared.

2. Methods

The rice straw sample (Ciherang variant) was harvested from Kampung Babakan Siliwangi, Desa Tambakmekar, Kecamatan Jalancagak, Subang, West Java.

The study was carried out by conducting pretreatment, enzymatic hydrolysis (saccharification), equalizing the concentration of the pure isolates yeast *S. cerevisiae* (control) with a Baker's yeast (F) and Tapai's starter [S], fermenting, measuring glucose levels, and analysizing glucose and ethanol levels in the sample. Pretreatment was conducted by cutting the fresh rice straw into strips of $\pm$1 cm, then boiled with distilled water for 1 hour, drained and dried out. Once dried, the samples were soaked using NaOH 1% (with the ratio 1:8 of NaOH 1%) for 4 hours, and filtered, then washed with distilled water until the pH reached neutral. Samples were dried using VWR Scientific 1370 GM at 65 °C for 2 days, and then blended to decrease the sample size to 10–20 mesh of screen [13].

The blended samples were hydrolyzed enzymatically (saccharification) by the enzyme mixture (cellulase). Samples were weighed analytically as much as 3% w/v [Precisa XT 220 A] and filled in 1,000 ml Erlenmeyer flask (Duran), then added 0.05M citrate buffer pH 5.2 and sterilized in an autoclave (HICLAVE™ HV-110) at 121 °C for 20 min. The mixture of sample and citrate buffer which had been sterilized was cooled in a laminar air flow (Esco Class II BC). Furthermore, 15 FPU/g cellulase enzymes (Celluclast) and 30 IU/g of β-glucosidase enzyme (Novozyme) were added into the mixture of sample and buffer citrate [14], then incubated using shaking incubator (Labtech ®) with 120 rpm at temperature of 50 °C for 108 h. The glucose level in the sample's hydrolyzate was checked using a glucometer (Accu-chek *Active*) then immediately stored in refrigerator at 4 °C [15]. Sugar reference was prepared in accordance with the data obtained from the glucose level in the sample's hydrolyzate, then sterilized and kept in a sterile and aseptic room [16].

The cell concentration of pure yeast isolates *S. cerevisiae*, F and S were equalized using the Turbidity method, wherein each inoculum was bred in sterile and aseptic Reader medium (compositions: Amonium sulfate $(NH_4)_2SO_4$ 8 g, $MgSO_4.7H_2O$ 1 g, KCl 0.3 g, $CaCl_2$ 0.1 g, *yeast extract* 10 g and glucose monohidrat 20 g to obtain 2000 ml of medium), then incubated for 24 hours in a shaking incubator at 120 rpm at 30 ^oC. The culture medium then was placed in an ice-filled container. Absorbance values of the cultured medium were measured using a spectrophotometer (Genesys 10 UV – Visible Spectrophotometer) with blank of medium Reader. Dry weight of the rest culture medium was calculated as follows: a number of predetermined volume was filled into 15 mL conical tube whose weight was

already known (Blue Max Jr. 17 x 120 mm). Then the conical tube was centrifuged at 4500 rpm for 10 minutes (Mikro 220 R Hettich Zentrifugen). The filtrate was discarded carefully from the tube, then conical tubes with residue were dried in an oven with a temperature of 105 °C for 2 hours (residues maintained so as not to scorch). Each conical tube containing the dried residue was weighed analytically, and the data was recorded. Standard curve of absorbance and dry weight values were derived. Concentration of cells obtained for fermention of sample's hydrolyzate was 2 g/L [17].

Treatment of solid F to the sample's hydrolyzate (PF) and sugar reference (GPF) was also performed. F was added into sample's hydrolyzate and sugar reference without being bred. The amount of solid F that was added into the substrate synchronized with 2 g/L concentration of F cells that had been cultured.

For the fermentation process, *S. cerevisiae*, F and S were growth up in the medium Reader, incubated for 24 hours in a shaking incubator at 120 rpm and temperature of 30 ^{o}C. The culture medium was transferred to the laminar air flow, and absorbance value was measured with a blank medium Reader. Measurements were repeated two times, and the average of absorbance values was calculated. The average value of absorbance was inserted into equations in order to obtain the volume of each culture medium that has a cell concentration of 2 g/L [16]. The volume then was multiplied by 8, and filled in a conical tube, centrifuged at 4 °C, RCF 1500 g for 5 min (Mikro 220 R Hettich Zentrifugen) [17]. Filtrate in conical tube was discarded carefully. The residue of each inoculum living cell in the conical tube was washed by re-centrifuging twice at 4 °C using sterile distilled water. To each conical tube containing residue was added sterile distilled water to obtain a total volume of 6 mL; this was then shaken until homogeneous stage was reached.

Fermentation was initiated by filling 0.75 mL of the total volume living cell of each conical tube into 24.25 ml of the sample's hydrolyzate and into sugar references that were already prepared in 200 mL sterile Erlenmeyer flasks. The mixture then was incubated in a shaking incubator at 120 rpm and temperature of 30 ^{o}C. Then 1.2 mL of fermented sample's hydrolyzate and sugar reference was taken periodically at 0, 4, 12, and 24, and filled into the microtube. All samples were stored in a freezer.

Glucose levels of each sample were measured using a glucometer. The data was recorded and a glucose trend curve was drawn (Fig. 1–3 in Results and Discussion). Bioethanol was analyzed using high-performance liquid chromatography (HPLC) in H column. Samples at t_1 (4 h) in the amount of 0.5 mL were withdrawn, diluted with 0.5 mL of H_2O in a 2 mL vial (Waters), and filtered through a high-performance liquid chromatography filter (Waters; OGS Healthcare syringe filter 1 mL/cc with a 0.22 µm MS ® CA Membrane Solution).

3. Results and Discussion

Equalization of cell concentration between pure yeast *S. cerevisiae* and both starters. Pure yeast *S. cerevisiae* was in cell form, but F was in powder form (granule) and S was in solid form. Since the inocula were in different forms, equalization was perform in order to carry out fermentation in the same conditions. Turbidity method was used to equalize *S. cerevisiae*, F, and S to obtain the same cell concentration of 2 g/L [17].

Glucose and bioethanol levels in rice straw hydrolyzate analyzed using high-performance liquid chromatography (HPLC). Glucose and bioethanol concentration were calculated by multiplying the area that shows the retention time of glucose (range 10.8 to 11.1) or ethanol (17.8 range) with the formula obtained from the calibration curve. Bioethanol production rate was obtained by dividing the concentration of ethanol (units of g/L) of the data with the point collection time t_1 (4 h).

Table 1 indicate that glucose levels are not directly proportional to the bioethanol produced in the treatment. F produces a higher glucose level (8.1 g/L) than S (7.7 g/L). However, the level of bioethanol produced by F is lower (4.8 g/L) compared with that produced by S (5.1 g/L). The ethanol production rate per hour of F is 1.2 g/L.h lower than that of S (1.3 g/L.h).

Fermentation of rice straw hydrolyzate using inoculum *S. cerevisiae*. The sample's hydrolyzate fermented using *S. cerevisiae* (control) at t_0 had a glucose level of 14 g/L, but showed lower glucose levels at t_1, t_2, t_3; 6.8 g/L, 5.77 g/L and 6.03 g/L, respectively. This pattern is similar to that of sugar reference. However, glucose levels from the sample's hydrolyzate using *S. cerevisiae* tended to remain stable up to t_3, while the glucose levels at sugar reference were likely to continue to decline.

S. cerevisiae is a yeast species that has a zymase enzyme that is able to ferment glucose into ethanol. Fermentation is known as a process to change sugars into ethanol compounds. The reaction will reduce glucose levels in the sample with increasing time. Futhermore, the concentration of ethanol tends to increase [18].

Fermentation of rice straw hydrolyzate and sugar reference using F inoculum. Glucose levels in the sample's hydrolyzate using F at t_0 is 13 g/L, but at t_1, t_2, and t_3 showed lower glucose levels of 6.17 g/L, 5.93 g/L, and 5.70 g/L, respectively. This pattern is similar to

the sugar reference. In rice straw hydrolyzate using F, glucose levels also remain stable up to t_3, while the sugar reference is likely continue to decline. This pattern is similar to the pattern of glucose levels in the use of *S. cerevisiae*, since F has the same inoculum colony.

Fig. 2 exhibits that samples (PF) that were given solid F directly showed a downward trend of glucose levels, but remained stable in sugar reference (GPF). The difference of glucose levels between PF and GPF is estimated to be due to additional nutrients contained in the rice straw hydrolyzate that drive solid F to grow and produce ethanol. The nutrients in question require further research.

Fermentation of sample's hydrolyzate and sugar reference using S inoculum. The glucose level in rice straw hydrolyzate using S innoculum at t_0 is 13.9 g/l, whereas at t_1, t_2, and t_3 glucose levels are lower, at 6.60 g/l, 6.50 g/l and 5.67 g/l, respectively. The same pattern occurred in the sugar reference. However, glucose levels remained stable up to t_3 at rice straw hydrolyzate, while the glucose levels of sugar reference tended to decline.

Table 1. Glucose and Bioethanol Concentration, and Bioethanol Production Rate from Rice Straw Hydrolyzate

Starter	Glucose concentration (g/l)	Bioethanol concentration (g/l)	Bioethanol production rate (g/l.h)
*Sc**	7.0	1.2	0.3
F	8.1	4.8	1.2
S	7.7	5.1	1.3
RS*c**	18.6	9.5	2.4
RF	17.2	12.8	3.2
RS	28.0	3.8	0.9

Sc = S. cereviseae

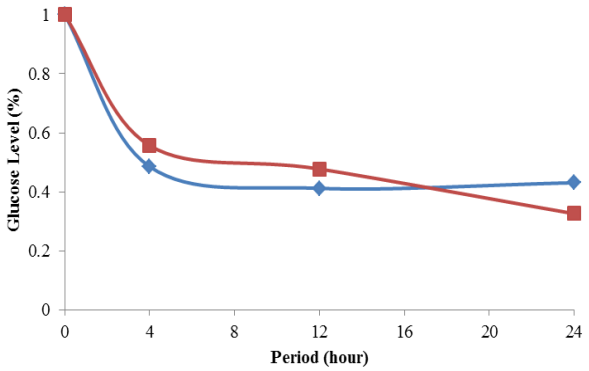

Figure 1. Assimilation Curve of Glucose Content in the Sample; Sc (♦) and Sugar Reference; GSc (■) Using *S. Cerevisiae*

Statistical analysis shows that there are significant differences in the inoculum (R, *S. cerevisiae*, PF, and PS), P-value is smaller than α (0.012 < 0.05). In other words, the R factor significantly affects glucose levels. Interaction of time factor (T; 0, 4, 12, and 24 hours) and R factor (TR) does not show a significant difference (P-value greater than α (0.103 > 0.05). In other words, the interaction factor TR has no significant impact on glucose levels.

Comparison of glucose consumption between pure isolates and yeasts. Fig.ure 4 exhibits the glucose levels' curve of the sample's hydrolyzate using *S. cerevisiae* (control), F, and S. The curve indicates that *S. cerevisiae*, F, and S used in the sample's hydrolyzate produce relatively similar amounts of glucose.

Comparison among inoculum (R) using the Games-Howell Test showed that glucose levels do not differ significantly. *S. cerevisiae* (Sc) and PF (F) P-value greater

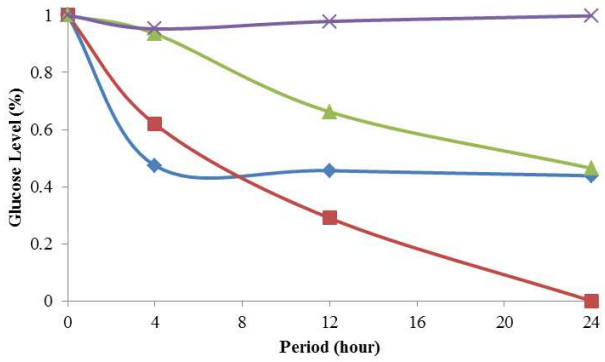

Figure 2. Glucose Assimilation Curve of Sample; F (♦), Sugar Reference; GF (■), F Solids Directly on the Sample; PF (▲), and Sugar Reference for PF; GPF (×)

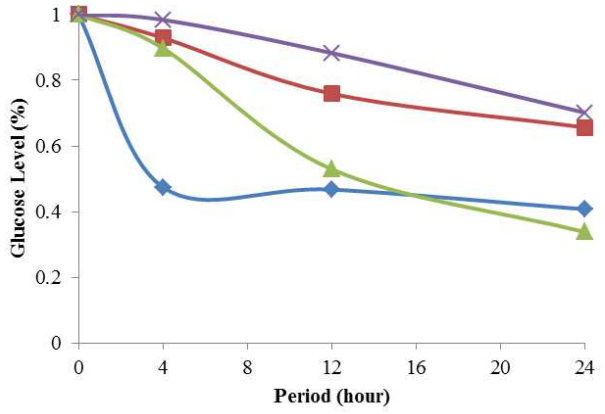

Figure 3. Glucose Assimilation Curve Sample; S (♦), Sugar Reference; GS (■), S Solids Directly on the Sample; PS(▲), and Sugar Reference; GPS (×)

than α (0.944 > 0.05). *S. cerevisiae* (Sc) and PS (S) P-value greater than α (1.000 > 0.05). PF (F) and PS (S) P-value greater than α (0.938 > 0.05).

Effectiveness of bioethanol production from rice straw hydrolyzate using F and S. Glucose levels in the rice sample's hydrolyzate using F and S show that both types of yeast contained high glucose levels at t_0 but decreased at t_1, and tended to be stable at up to t_3 at an average of 6.2 g/L (F) and 5.93 g/L (S). High levels of glucose on t_0 are due to no glucose consumption since incorporation of inoculum into the substrate. Glucose levels become lower in t_1 because the inoculum has grown and consumed the glucose.

The more colonies of *S. cerevisiae*, the lower the glucose levels, due to inoculum consumption; however, in t_2 and t_3 the glucose levels are stable as in t_1. *S. cerevisiae* has a threshold of life in alcohol; the more alcohol that is produced in the fermentation process, the higher colony of *S. cerevisiae* will die [19]. Amerine *et al*. stated that the fermentation process resulting volatile acids, such as lactic acid, acetic acid, formic acid, butyric acid and propionic acid. The higher the glucose level, the higher the amount of acid, thus the higher acidity and pH decrease of the substrate [20]. Glucose levels at t_1, t_2, t_3 are likely to remain stable, showing that *S. cerevisiae* has reached the threshold of life and consumption of glucose. Consequently, bioethanol production is stagnant. This result suggests that future research on the fermentation substrate for both types of yeast do enough up to t_1 or in 4 h.

Starter S shows higher bioethanol production compared to F, although statistically it is not significantly different. Starter S also shows better effects in the rate of production of bioethanol (Table 1). Starter S contains more microbes (yeasts, molds, and bacteria), resulting in a higher production of bioethanol than F, which contains only *S. cerevisiae*. Fermentation is a process of gradual change with the help of microorganisms, i.e. yeasts, molds, and bacteria [21]. According to Sondari *et al*. [19], a growing number of yeasts and bacteria present in the process of yeast fermentation is optimized, so more alcohol is produced. The research represents PS on bioethanol production from rice straw. The other advantage of using PS on bioethanol production is that it is cheaper and easier to find at any traditional market.

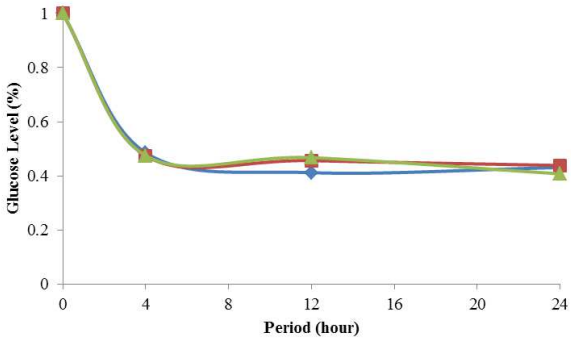

Figure 4. Comparison of Glucose Levels in Sample's Hydrolyzate Using *S. cerevisiae* (♦), F (■), and S (▲)

Table 2. Processing of Rice Straw Waste into Bioethanol

	West Java	Indonesia	National Petrol Substitution
Rice Production (kg) [22]	11,633,891,000	69,045,141,000	
Rice Straw (Kim & Dale) (kg) [8]	16,287,447,400	96,663,197,400	
Bioethanol (F) (l)	61,924,875	367,513,477	268,284,838
Bioethanol (S) (l)	66,061,887	392,065,929	286,208,128
Bioethanol (Kim & Dale) (l)	4,560,485,272	27,065,695,272	
Bioethanol (Badger) (l)	3,257,489,480	19,332,639,480	
Comparison of Kim & Dale with F	74		71x lower
Comparison of Kim & Dale with S	69		
Comparison of Badger with F	53		51x lower
Comparison of Badger with S	49		

Rice straw potential as raw material of bioethanol. The study shows that the hydrolyzate of each 15 gram (3% w/v on methodology) dry weight of straw obtained 4.8 g/L bioethanol if fermented using F, and 5.1 g/L bioethanol if fermented using S (Table 1). Each 15 gram dry weight of straw materials is equivalent to 40 grams of field rice straw. Thus, every 1000 g of field rice straw obtains bioethanol between 120 g/L (F) and 127.5 g/L (S). In other words, bioethanol produced from rice straw is between 3.802 mL/kg (F) up to 4.056 mL/kg (S).

If the research results converted to rice straw production in West Java as regional representative of materials samples, and the production of rice straw nationally as a whole, the bioethanol obtained would be as listed in Table 2. It appears that bioethanol production from rice straw for West Java is between 61,924,875 L and 66,061,887 L, whereas nationally it is between 367,513,477 L and 392,065,929 L. Processing agricultural waste rice straw into bioethanol could be one of the solution to mitigate the fuel crisis by substituting for gasoline up to 45,205,159 L in West Java, and up to 288,284,838 L nationally. The result is much lower (about 71 times and 51 times, respectively) compared to the production analysis by Kim & Dale or Badger. This evidence indicates that more research should be conducted.

Utilization of waste rice straw into bioethanol production would not only enable us to cope with the fuel crisis by serving as a source of alternative energy, but would also generate a positive effect on reduction of waste by preventing pollution caused by the waste rice straw.

4. Conclusions

The effects of both types of yeast on the production of bioethanol content of the samples was not significantly different. However, starter S (solid starter) produced higher bioethanol levels (5.1 g/L) compared to that of F (powder starter) (4.8 g/L). The rate of bioethanol production of S is also higher (1.3 g/L.h) than that of F (1.2 g/L.h). S is recommended in bioethanol production from rice straw hydrolyzate due to its low-priced availability. Further research is needed to increased the yield of bioethanol production from rice straw hydrolyzate using starter S (Tapai's starter) in the household scale in order to be able to apply directly to the public.

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