Scientific Papers. Series D. Animal Science. Vol. LVI
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN-L 2285-5750

HYDROLYSIS OF SAGO PITH POWDER (METROXYLON SAGO ROTTB.) IN ENZYMATIC AND FERMENTATION OF HYDROLYZATE BY *PICHIA STIPITIS CBS 5773, SACCHAROMYCES CEREVISIAE D1/P3GI,* AND *ZYMOMONAS MOBILIS FNCC 0056* TO ETHANOL

Ratu SAFITRI, Abi WIYONO

Lab. of Microbiology, Dept. of Biology, Faculty of Mathematics and Sciences,
Padjadjaran University, Jalan Raya Bandung-Sumedang Km. 21 Jatinangor Sumedang 45363,
Bandung - West Java, Indonesia

Corresponding author email: ratusafitrie@yahoo.com

Abstract

Sago (Metroxylon sago Rottb) is an abundant source of biomass and have a high starch content, which can be used as a source of renewable energy (bioethanol). The purpose of this study is to obtain the best microorganisms to ferment sugars of the enzymatic hydrolysis yield of sago pith into bioethanol. The research method is experimental in the laboratory consisting of starch hydrolysis and fermentation of sugar pith of sago pith of sago starch hydrolyzate by Pichia stipitis CBS 5773, Saccharomyces cerevisiae D1/P3GI, and Zymomonas mobilis FNCC 0056 in single culture. Pith of sago flour used in this study contained 77.5% starch, 4.86% hemicellulose, cellulose 4.63%, 3.07% lignin and 10.12% water. Through gelatinization and enzymatic hydrolysis of sugar, the sugar concentrations obtained in this study was 47.95% and 61.66% DE. Hydrolysates were used containing 10% sugar, fermented ethanol showed that ethanol production by Pichia stipitis of 2.45% without pH adjustment, 2.19% with a pH adjustment treatment, the concentration of ethanol by Saccharomyces cerevisiae at 3.65% without adjusting the pH, 2.17% with a pH adjustment, and the concentration of ethanol by Zymomonas mobilis at 1.89% for those without pH adjustment and 0.37% with a pH adjustment.

Key words: *Ethanol, Hydrolisis, Sago Palm Pith Powder, Fermentation, Pichia stipitis CBS 5773, Saccharomyces cerevisiae D1/P3GI, Zymomonas mobilis FNCC 0056.*

INTRODUCTION

Pith of sago starch has a complex structure, composed of cellulose, hemicellulose, and starch. Starchy pith is also difficult to dissolve in water because it contain amylopectin more than amylose (Hidayat et al., 2006).

Cellulose is a long polymer of D-glucose and has a very regular shape with ß-1, 4 glycosidic bonds. Cellulose hydrolysis yields D-glucose relatively large (Lehninger, 1997). Unit composed of hemicellulose sugars (sugar anhidro) which can be divided into groups of pentose, hexose, hexuronat acid and deoxy-hexoses. Hydrolysis of hemicellulose in particular are composed of hexose sugar units also produce simpler compounds of ß-D-glucose and D-galactose (Fengel, 1995).

To produce bioethanol from stem pith of sago starch it is necessary to do gelatinization. The purpose of gelatinization is to open up the structure of lignocellulose so that the cellulose can be hydrolyzed by the enzyme.

After starch, cellulose and hemicellulose is hydrolyzed to C6 and C5 sugars then fermented into ethanol. Hemicellulose is a heterogeneous polysaccharide, it can be produced relatively easy to hydrolyze into sugar hexoses and pentose sugars that ready to be fermented into ethanol. Hexose sugars include glucose, mannose, galactose, and a little ramnosa. While the pentose sugars include xylose and arabinose (Taherzadeh and Karimi, 2007). Thing that Starch, cellulose, and hemicellulose have in common is they composed of simple sugars D-glucose which ready to be fermented by microorganisms to produce ethanol (Said, 1987).

Enzyme hydrolysis aims to hydrolyze cellulose and hemicellulose into sugar monomers. Enzymatic hydrolysis can be carried out using a hydrolase enzyme a-amylase enzyme, hemicellulase, cellulase, and amiloglucosidase (Gerhartz, 1990). Enzyme hydrolysis process is divided into three stages; the first is the

liquefaction, the liquefaction process pith of sago starch gel using the enzyme a-amylase (Judoamidjojo et al., 1992). The second stage is the hydrolysis of hemicellulose using hemicellulase enzymes. Purpose of hemicellulase enzymes is to convert hemicellulose into simple sugars units in the form of units of hexose sugars and pentose sugar unit. Then proceed with saccharification, which is split into glucose likuifikasi results using cellulase enzymes and enzyme amiloglucosidase (Judoamidjojo et al., 1992).

Stem pith of sago starch hydrolysis using α-amylase, hemicellulase, cellulase, and amiloglucosidase. Based on the previous research Flour pith of sago starch content was found to have 77.5%, 4.63% cellulose, 4.86% hemicellulose, lignin 3.07%, and 10.12% water. Starch hydrolysis process enzymatically provide value of DE (Dextrose Equivalent), at around 95 percent (Judoamidjojo et al., 1992). Hydrolysis of hemicellulose to produce two types of sugar, which is sugar pentose and hexose sugars. The resulting hexose sugars are glucose, galactose, and mannose, while the resulting pentose sugar is xylose and arabinose (Taherzadeh and Karimi, 2007).

Process of ethanol fermentation using fermentative microorganisms influenced by pH condition and the concentration of simple sugars. P. stipitis can grow well at pH 4.0 to 7. *S. cerevisiae* can survive at pH 4.0 to 7.0 (Marx, 1991), while *Z. mobilis* can grow well at pH 6.0 to 7.0 (Gunasekaran et al., 1986).

According Obire (2005) pH range for *S. cerevisiae* is optimum at pH 3-7 and 5, to *P. stipitis* has an optimum pH range of 4-7 and at pH 5, and to *Z. mobilis* has an optimum pH range of 4.5 to 7 and at pH 7. Glucose fermentation using *Saccharomyces cerevisiae* to produce high concentrations of ethanol, tolerant of high concentrations of alcohol [12-18% (v / v)], resistant to high sugar levels and remain active in the fermentation temperature 4-320C. While *Zymomonas mobilis* is superior because, it is more resistant to high temperatures and acidic pH (Aziz, 2002). Based on the Haagensen (2005) research, *Saccharomyces cerevisiae* only capable to fermenting hexose sugars, while *Pichia stipitis* could ferment pentose sugars Haagensen (2005) xylose. Productivity and the amount of ethanol produced by P. stipitis xylose fermentation is greater than *S.cerevisiae*. *Z.mobilis* is a type of bacteria that can produce ethanol. As with *S. cerevisiae*, these bacteria can only convert hexose sugars to ethanol, but the ability fermentation is faster than *S. cerevisiae* (Davis et al., 2006).

MATERIALS AND METHODS

Materials

The usedequipments were HPLC (High Performance Liquid Chromatography), shakers, spectrophotometers, Stopwatch. The materials used in this study are the stem pith of sago starch (sago Metroxylon Rottb.), Culture *Pichia stipitis* CBS 5773, *Saccharomyces cerevisiae* D1/P3GI, and *Zymomonas mobilis* FNCC 0056. The enzyme a-amylase liquozyme supra, dextrozyme amiloglukosidase enzyme, the enzyme hemicellulase (Sigma), Sigma cellulase enzyme (Novozyme).

Methods

The method used in this research is descriptive like experimental methods in the laboratory. This research is generally divided into two stages, the stem pith of sago starch hydrolysis into reducing sugars and fermentation of hydrolyzate into ethanol formed. Sago trunk in the process in the form of powder size of 100 mesh. Tepung in gelatinization by stem pith of sago starch in the suspension and then incubated at a temperature of 120^{o}C for 20 min with a pressure of 1 atm. Subsequently the pH was adjusted to 6.0 using a solution of Hydrochloric Acid (HCl) and 1 N sodium hydroxide (NaOH) 1 N. The enzyme a-amylase is added at 0.17 mL / g (volume enzyme / g substrate). After it was incubated at 104^{o}C for 60 min with a pressure of 1 atm.

The results obtained are first measured the concentration of sugar hydrolyzate and dextrose equivalent (DE). I hydrolyzate is heated at a temperature of 121^{o}C with a pressure of 1 atm for 10 minutes. Hemicellulase enzyme is then added as much as 1/3 dose. Hydrolyzate subsequently incubated at 55^{o}C with agitation at 150 rpm for 270 minutes. Then the measured parameter is the concentration of reducing sugar and dextrose equivalent (DE). Hydrolyzate has cooled to 25^{o}C temperature. Then the pH was adjusted to 4.8 using 1 N HCl and NaOH 1 N.

Followed by the addition of cellulase enzymes as much as 0.55 mL / g and 0.37 mL enzyme as much amiloglucosidase / g (volume enzyme / g substrate). Subsequently incubated at 60°C for 48 h with agitation speed of 130 rpm.

At this stage, the measured parameter is the concentration of reducing sugar. Before entering the stage of fermentation, and cellulase enzyme used amiloglukosidase heated at 100°C for 10 minutes (Gerhartz, 1990). Sugar hydrolyzate saccharification results then set to a concentration of 10% and 20%. Each hydrolyzate plus sugar fermentation medium containing (per liter): yeast extract 4 g, 2 g KH_2PO_4, $(NH_4)_2SO_4$ 3 g, 1 g $MgSO_4.7H_2O$, and pepton 3, 6 g (Sanchez et al., 2002). After the substrate pH adjusted using HCl 0.83 N and 0.83 N NaOH. For fermentation by *P. stipitis* CBS 5773 and *S. cerevisiae* D1/P3GI made pH 5 medium, and fermentation by *Z. mobilis* 0056 FNCC made medium pH 7. Fermentation substrate sterilized in an autoclave at a temperature of 1210°C for 15 min with a pressure of 1 atm. The parameters used were: concentration of total sugar concentration, Dextrose, bacterial cell number, pH and the concentration of ethanol.

RESULTS AND DISCUSSIONS

Enzymatic Hydrolysis of Sago Pith Flour

Hydrolysis process begins with gelatinization, is a mechanism of entry of water into the starch granules that will facilitate the work of the hydrolyzing enzyme substrate.

Table 1. Sugar DE (Dextrose Equivalent) value of Hydrolysis Enzymatically of Sago Pith Flour

Enzyme	Cons. Of Sugar (%)	DE
Alpha amylase	40.75	52.41
Hemicellulase	42.69	54.90
Amyloglucosidase + cellulase	47.95	61.66

Alpha-amylase break down amylose molecules produce glucose and maltose, into the amylopectin, work of this enzyme produces glucose, maltose, and dextrin. Based on reducing sugar measurement results obtained DE value of 52.41%. Further provision of hemicellulase, hydrolyze hemicellulose into glucose and xylose (Howard et al., 2003), and the obtained value of 54.90% of DE.

Giving of cellulase will convert cellulose into glucose, while amyloglucosidase convert amylose and amylopectin into glucose monomers, since this enzyme is able to break the bonds of alpha-1,4 glycosidic and 1,6 alpha-glycosidic so dextrin become glucose and at this stage DE values generated by 61.66%. Increase in value due to Dextrozyme DX DE, breaking the glycosidic bond α-1, 4 and α-1,6 Dextrozyme DX, is a combination of amyloglucosidase, and pullulanase so the process of bond breaking α-1,6-glycosidic become faster and more effective. Overall, the pith of sago starch hydrolysis enzyme producing reducing sugar concentration of 47.95% with a DE value of 61.66%, its mean that there is about 38.34% has not been hydrolyzed.

Fermentation of sugar hydrolizate of Sagu pith Flour by *Pichia stipitis* CBS 5773 *Saccharomyces cerevisiae* D1/P3GI, and *Zymomonas mobilis* FNCC 0056

pH of Fermentation

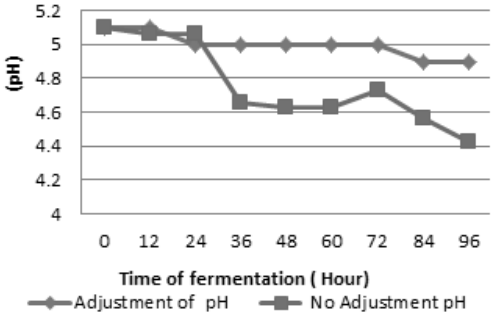

Figure 1. Changes in pH during fermentation by Pichia stipitis

Figure 1 showed that during the fermentation process, the pH has decreased; due to the formation of acid compounds results byproducts of metabolism. Initial acidity of the fermentation medium is 5, where the pH is an optimum pH for growth of *P. stipitis* for growth processes. Treatment pH adjustment is done to maintain optimum conditions for the growth of *P. stipitis*, where the pH is maintained between 4.8 to 5 settings. Whereas untreated pH adjustment pH decline, which was initially 5.1 to 4.43, this means that the pH has decreased by 13.1%.

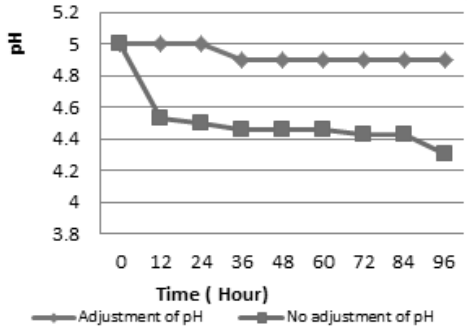

Figure 2. Changes in pH During Fermentation by Saccharomyces cerevisiae

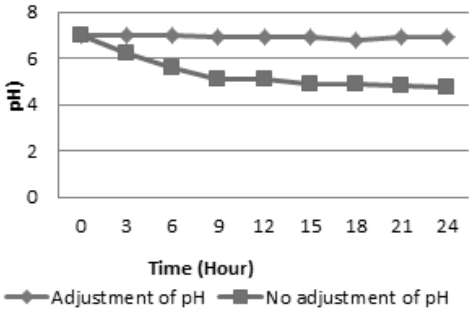

Figure 3. Changes in pH During Fermentation by Zymomonas mobilis FNCC 0056

On fermentation by *S. cerevisiae* of this study, the pH of the fermentation medium is set by 5, due to a pH range of 4-7 pH optimum (Obire, 2005). During the 96 hours fermentation pH decreased from the initial pH of 5.0 to 4.3, however, the pH was produced the optimum pH range. PH changes during fermentation by *Z. mobilis* down from pH 7 to 4.76, a decrease in pH by 42%. The decrease in pH due to *Z. mobilis* produce lactic acid as a product of metabolism (Buchanan and gibbons, 1994). Changes in pH caused by fermentation substrate that cells use ammonia as a nitrogen source is converted into NH4+. NH4+ molecules will merge into the cell as R-NH3. In this process H + left in the media, so that more biomass and fermentation time increased H^+ ion is causing more and more in the substrate which causes the lower the pH of the media (Fardiaz, 1988).

Fermentation ability of *P. stipitis*

Fermentation medium with initial sugar concentration of 10% containing 8.34% glucose and 1.20% maltoheptase. Changes in sugar concentration and ethanol concentration during fermentation can be seen in the following figure 4.

pH treatment adjustment effect the pattern of sugar consumption by microorganisms, fermentation with pH adjustment is able to consume more sugar than without pH adjustment, this is because the growth is at the optimum pH range is between 4-6.4-6. Sugar concentration for pH adjustment treatment, down from 8.41% to 2.68%, resulting in a decrease of 68%. While fermentation without pH adjustment only sugar concentration decreased by 55.2%, with 8.34% initial sugar concentration drops to 3.73%. The decrease in the concentration of sugar because sugar is used for metabolism can also be due to the low pH of the medium .

Efficiency of fermentation by *P. stipitis* with pH adjustment is smaller than that without pH adjustment, pH adjustment to the treatment efficiency of fermentation by 26% and for the culture without pH adjustment treatment efficiency of fermentation by 29.3%. Value fermentation efficiency by *P. stipitis* is low due to its ability to ferment glucose is very low, because the best is the ability to ferment pentose sugars, namely xylose (Almaeda et al., 2008).

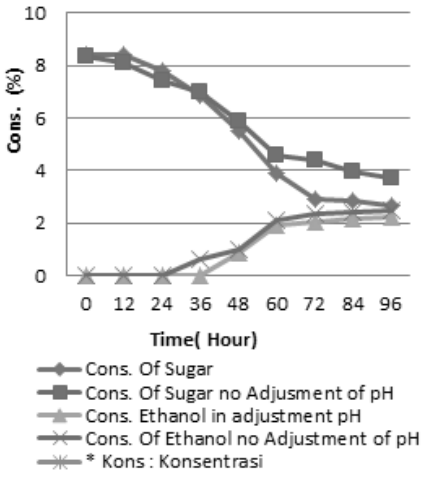

Figure 4. Concentrations of Sugar and Ethanol During Fermentation by P. stipitis

Result of lower concentrations of ethanol produced during fermentation is also due for

the addition of NaOH pH adjustment, and led to an influx of oxygen into the bottle fermentation, so it can affect the stability of the fermentation process, the ethanol fermentation process when it should be in a state of anaerobic (Almaeda et al., 2008).

Ability ethanol fermentation by *Saccharomyces cerevisiae* D1/P3GI. Fermentation medium with initial sugar concentration of 10% containing 8.48% glucose and 1.52% maltoheptaosa. Percentage of sugar concentration and ethanol produced during fermentation (96 hours) by *S. cerevisiae* by treatment setting and without pH adjustment can be seen in Figure 5.

Concentration of ethanol produced in fermentation by *S. cerevisiae* without adjusting the pH at 3.65%, and the pH adjustment of 2.17%. Efficiency ethanol fermentation by *S. cerevisiae* without pH adjustment of 43.3%, whereas the treated fermentation pH adjustment has an efficiency of 25%. According Periyasamy et al (2009) states that the maximum ethanol that can be produced in theory is 51%, meaning that 1 gram of glucose can produce 0.51 grams of ethanol. *S. cerevisiae* produces a maximum of 53% ethanol with optimum conditions, at pH 4 and temperature of 35^{o}C.

No fermentation efficiencies up to 51% due to the formation of acetic acid, lactic acid, and the formation of heat and steam, thus inhibiting *S. cerevisiae* to produce ethanol, while the lower fermentation efficiency by adjusting the pH due to the addition of sodium hydroxide, so that it can inhibit the growth and affect the concentration of ethanol produced (Rahman, 1992).

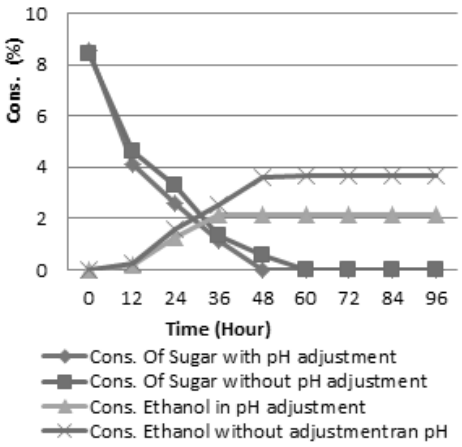

Figure 5. Concentrations of Sugar and Ethanol During Fermentation by Saccharomyces cerevisiae D1/P3GI.

Ethanol Fermentation Capabilities By *Zymomonas mobilis*.

Initial concentration of reducing sugar used in the fermentation of glucose is composed of 8.13% glucose and 1.87% maltoheptase. Consumption pattern of sugar fermentation showed the pH adjustment is able to consume more sugar than without pH adjustment. Sugar concentration during the final 24 hours of fermentation by *Z. mobilis* can be seen in Figure 6.

During fermentation sugar concentration decreased by 72.1%, the initial sugar concentration of 8.13% to 3.37% in the fermentation without pH adjustment, and with pH adjustment in sugar consumption by 58.5% where the initial sugar concentration amounted to 8.22% down to 2.29%. According Obire (2005), *Z. mobilis* not able to ferment the sugar arabinose and maltose, arabinose contained in the medium is only used to add biomass to produce ethanol instead, *Z. mobilis* is a bacterium that has the rate of glucose consumption, ethanol production and a high tolerance to ethanol.

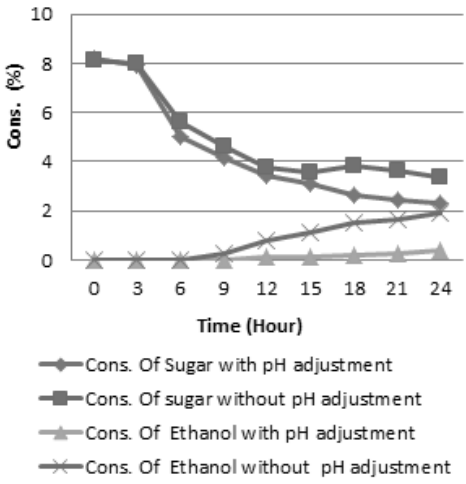

Figure 6. Concentrations of Sugar and Ethanol During Fermentation by Z. mobilis

CONCLUSIONS

Saccharomyces cerevisiae D1/P3GI is best microorganisms to ferment sugars in the enzymatic hydrolysis results pith of sago starch to ethanol with no pH adjustment is obtained with a concentration of 3.65% ethanol and fermentation efficiency of 43.3%, while the pH adjustment with the highest concentration of ethanol produced by *Pichia stipitis* CBS 5773 by 2.91% with a fermentation efficiency of 29.3%.

pH adjustment provides increased consumption of sugar, but the produced ethanol concentrations did not differ with fermentation without pH adjustment.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Ministry High of Education through Competition Grant Scheme.

REFERENCES

Almaeda J.R., T. Modig, A. Roder, G. Liden, M.F.G. Grauslund, 2008. Pichiastipitis Xylose Reductase Helps Detoxifyng Lignocellulosic Hydolysate by Reducing 5 Hydroxymethyl Furfural (HMF). Biotechnology for Biofuels. 12(10), p. 1-12.

Aziz S. A., 2002. Sago Starch and Its Utilization. Journal of Bioscience and Bioengineering. Vol. 94. No. 6. Hal, p. 526-529.

Buchanan R.E., N.E. Gibbons, 1994. Bergey's manual Of Determinating Bacteriology Edisi IX. London: The Williams & Wilkins Company.

Davis L., P. Rogers, J. Pearce, P. Peiris, 2006. Evaluation of Zymomonas-Based Ethanol Production From a Hydrolysed Waste Starch Stream. Elsevier Biomass and Bioenergy. 30, p. 809-814.

Fardiaz S., 1988. Fisiologi Fermentasi. Bogor: Pusat Antar Universitas IPB bekerja sama dengan Lembaga Sumber Daya Informasi IPB.

Fengel D., G. Wagener, 1995. Kayu Kimia Ultrastruktur Reaksi-Reaksi. Yogyakarta : Gadjahmada University Press.

Gerhartz W., 1990. Enzymes in Industry Production and Applications. Weinheim: VCH Verlagsgesellschaft mbH.

Gunasekaran P., T. Karunakaran, M. Kasthuribai, 1986. Fermentation Pattern of ZymomonasmobilisStrains On Different Substrates—a Comparative Study. J. Biosci.10 (2), p. 181-186.

Hidayat N., M.C. Padaga, S. Suhartini, 2006. Mikrobiologi Industri. Yogyakarta : ANDI.

Haagensen F. D., 2005. Enzymes For Biomass And Forestry. http://www.novozymes.com.

Howard R.L., P. Masoko, E. Abotsi, 2003. Enzymeactivity of Phanerochaete chrysosporium Cellobiohydrolase (CBHI.1) Expressed as a Heterologous Protein From Escherichiacoli. African J. Biotechnol. 2(9), p. 296-300

Judoamidjojo M., Abdul A. Darwis, Endang G. Sa'id, 1992. Teknologi Fermentasi. Jakarta: Penerbit Rajawali.

Lehninger A.L., 1997. Dasar-Dasar Biokimia. Jakarta : Erlangga.

Marx J.L., 1991. Revolusi Bioteknologi. Jakarta: Yayasan Obor Indonesia.

Obire O., 2005. Activity of Zymomonas species in palm-sap obtained from three areas in Edo State, Nigeria. J. Appl. Sci. Environ.9, p. 25 – 30.

Periyasamy S., Sivakumare V. Sridhar R., Venkatesan S., 2009. Production of Bioethanol from Sugar Molasses Using Saccharomyces cerevisiae. Modern Applied Science vol 3, no 8.

Rahman A., 1992. Teknologi Fermentasi Industrial II. Jakarta: Penerbit ARCAN

Sa'id E.G., 1987. Bioindustri: Penerapan Teknologi Fermentasi. Jakarta: Penerbit Mediyatama Sarana Perkasa.

Taherzadeh M.J., K. Karimi, 2007. Acid-Based Hydrolisis Processes For Ethanol From Lignocellulosic. Bioresources. 2(3), p. 472-499.