Thesis for the Degree of Master of Engineering

Studies of fermentation process and recombinant yeasts to improve bioethanol productivity

by

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(바이오에탄올 생산성 향상을 위한 발효 공정과 재조합 균주에 관한 연구)

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바이오에탄올 생산성 향상을 위한 발효 공정과 재조합 균주에 관한 연구

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요약

화석연료 고갈, 이산화탄소(CO_2) 축적 등 환경 문제로 인해 대체에너지 개발 필요성이 대두되는 가운데, 친환경적이고 지속적인 에너지 생산이 가능한 바이오 에탄올이 전망되고 있다. 특히 바이오 에탄올 생산에 사용할 수 있는 자원으로서 해조류 바이오매스는 육상계 바이오매스의 한계를 극복할 수 있다는 점에서 주목되고 있다. 또한, 해조류를 이용한 바이오에탄올 생산 및 자원화를 통해 국내자급이 가능한 비식용성 해조류 자원화로 국가 대체에너지 보급률 확대에 이바지할 수 있을 것이며 국내 신재생에너지 생산 및 공급분야의 활용에도 기여하고자 한다. 따라서 본 연구는 해조률 기질로 하여 혼합당을 이용한 적응진화, 상업적 효소를 이용한 당화, 발효 공정 그리고 CRISPR Cas9 등으로 균주를 개발한 뒤 고농도 에탄올 생산 공정기술을 획득하고자 한다.

바이오매스로 해조류인 홍조류 *G. furcata*를 이용하여 바이오에탄올을 생산하였다. 10% (w/v)의 슬러리 농도, 염산(HCl) 농도 300mM, 처리 시간 90분 동안 121℃ 로 열산 가수분해(Acid hydrolysis)를 진행한 후, 16 units/mL의 상업적 효소를 사용하여 효소당화를 진행하였고, 최대 44.3 g/L의 단당을 생산하였다. 이로부터 에탄올 발효 생산 효율에 대하여 *S. cerevisiae*의 glycerol pathway에 관여하는 *GPD1*과 *GPD2*의 유전자를 각각 조작하였다. 이 재조합 균주를 이용한 발효를 통해 glycerol의 변화에 따라 glucose, galactose 그리고 ethanol 생산수율에 있어서 변화가 있는 지에 대해 비교하였다.

1. Introduction

Fossil fuel depletion and increased energy consumption have led to a greater interest in biofuel production. Bioethanol is a renewable biofuel that can reduce greenhouse gas emission and will burn completely compared with gasoline fuel. Recently, bioethanol has been used as a petroleum substitute for transportation.

Seaweeds, especially red seaweed, have substantial potential as a sustainable bioethanol-producing biomass source due to zero lignin content, rapid growth, non-arable land usage, and high carbohydrate content. The red seaweed *Gloiopeltis furcata* has 60 - 70% of carbohydrate content.

The carbohydrates in *G. furcata* are fractionated to yield monosaccharides through dilute acid hydrolysis and enzymatic saccharification. Dilute acid hydrolysis deconstructs the biomass to release monosaccharides. The most important parameters that can have an effect on dilute acid hydrolysis include the hydrolysis temperature, residence time, and pH, which may be represented by the combined severity factor. The hydrolysis of biomass using an acid could improve enzyme accessibility. The performance of enzymatic saccharification depends on the substrate type, enzyme activity, enzyme binding, and operating conditions. Furthermore, the addition of surfactants to the enzyme could improve cellulase activity and enzyme stability in enzymatic saccharification.

In *S. cerevisiae* cells, glycerol is synthesized from the reduction of

dihydroxyacetone phosphate, which is a glycolytic intermediae in two sequential steps catalyzed by the rate-limiting NAD+-dependent glycerol-3-phosphate dehydrogenase and a secondary reaction catalyzed by glycerol-3-phosphatase. *GPD1* and *GPD2* encode two isoenzymes of glycerol-3-phosphate dehydrogenase. The main role of glycerol is to balance the intracellular redox and adjust the osmotic stress within the cells.

The purpose of this work was to study the effect of *GPD1* and *GPD2* deletion on the production of glycerol and ethanol.

The optimal hyper thermal acid hydrolysis condition with a suitable combined severity factor was determined in order to obtain a high concentration of monosaccharides with a low level of inhibitors in the *G. furcata* hydrolysate. Furthermore, the effect of Tween20 with Cellic CTec2 on enzymatic kinetic was studied using the Michaelis-Mentenequation to improve the efficiency of enzymatic saccharification. The enhancement of glycerol and galactose consumption was conducted through the deletion of *GPD1*, *GPD2*, both *GPD1* and *GPD2* using the Cas9 system. The effects of engineered genes on transcriptionlevels of *GPD* and other repressor genes were analyzed by quantitative reverse transcription polymerase chain reaction (qRT-PCR) to determine the efficiency of glycerol and galactose consumption by the engineered strain compared with the control strain.

2. Materials and Methods

2.1. Raw materials

G. furcata was obtained from Wan-do, Jeonmam, Korea. The dried seaweed was ground using a hammer mill, and the resulting powder was passed through a 200-mesh sieve before experiment. The carbohydrate, crude fiber, crude protein, crude lipid, and ash contents of the seaweed were analyzed as described previously AOAC method.

The wild type *S. cerevisiae* CEN-PK2 (MATa; HIS3D1; LEU2-3_112;URA3-52; TRP1-289; MAL2-8C; SUC2) and the plasmid of pRS42H was obtained from Kyungpook National University. *S. cerevisiae* CEN-PK2 served as the host for the transformation of the CRISPR-Cas9 system.

2.2. Thermal acid hydrolysis

Optimization of thermal acid hydrolysis conditions was carried out with an 8 - 14% (w/v) seaweed slurry, 100 - 500 mM H_2SO_4, HNO_3 and HCl, and 15-120 min autoclaving time. Thermal acid hydrolysis was per-formed using 100 mL seaweed slurry and acids in a 250 mL Erlen-meyer flask. Seaweed slurry hydrolysates were then neutralized to pH 5.0 with 10 N NaOH. Samples were taken periodically and centrifuged. The supernatants were analyzed for mono sugars using HPLC. The efficiencies of thermal acid hydrolysis pretreatment was calculated as follows Eq. (1).

$$E_p = \triangle S_g / TC \times 100 \qquad \text{Eq. (1)}$$

in which E_p is efficiency of thermal acid hydrolysis pretreatment (%), $\triangle S_g$ is monosaccharides increase (g/L) during thermal acid hydrolysis pretreatment, TC is total carbohydrate (g/L) in pretreated *G. furcata*.

2.3. Enzymatic saccharification

Saccharification was conducted by adding 16 U/mL of ViscozymeL (1.2 FBG/mL, beta-glucanase, Novo-zymes, Bagsvaerd, Denmark), 16 U/mL Celluclast 1.5 L(8.4 EGU/mL, endo-glucanase, Novozymes) and Cellic CTec2 to 10% of seaweed slurry. Samples were taken periodically and centrifuged. The supernatants were analyzed for mono sugars using HPLC. The efficiencies of enzymatic saccharification and ethanol yield were calculated as follows Eq. (2).

$$E_s = \triangle S_g / C \times 100 \qquad \text{Eq. (2)}$$

in which E_s is efficiency of enzymatic saccharification (%), $\triangle S_g$ is glucose increase (g/L) during enzymatic saccharification after the pretreatment, C is cellulose content(g/L) in pretreated *G. furcata*.

2.4. Removal of HMF Enzymatic saccharification

HMF removal from the hydrolysate was performed using activated carbon powder (Duksan Pure Chemical Co., Ltd., Ansan, Korea). The 100 mL of hydrolysate with the addition of 0 to 5 % (w/v) the

activated carbon was placed in a shaking water bath at 150 rpm and 50 °C for 2 min of adsorption times. The adsorption surface areas of the activated carbon powder were 1400-1600 m2/g. After the adsorption, the activated carbon was removed by centrifugation (1390×g, 10 min) and the recovered supernatant was analyzed for monosaccharide and residual HMF. The efficiency of HMF removal (E_R, %) by the activated carbon was calculated as the removed HMF concentration (g) relative to the initial HMF concentration (g) as shown in Eq. (3).

$$E_R(\%) = \frac{C_{HMF} - R}{C_{HMF}} \times 100 \qquad \text{Eq. (3)}$$

where C_{HMF} is initial HMF concentration (g/L) and R is the concentration of remained HMF (g/L).

2.5. Yeast and selection marker

Wild type *S. cerevisiae* CEN.PK2 (MATa; HIS3D1; LEU2-3_112; URA3-52; TRP1-289; MAL2-8C; SIC2) was obtained from Kyungpook National University to serve as a host for the transformation of the CRISPR/Cas9 system. The pRS42H-*GPD1* and pRS42H-*GPD2* plasmids were transformed in *Escherichia coli* DH5α, with hygromycin B used as selective markers for pRS42H-*GPD1* and pRS42H-*GPD2*, respectively.

2.6. GPD1 and GPD2 deletion using CRISPR Cas9

The *S. cerevisiae* genome was engineered with a clustered regularly interspaced short palimdromic repeat (CRISPR)/Cas-9 system and primer designs according to the method of KIM et al. Guide RNA (gRNA) was designed with 20 bp of a *GPD1* or *GPD2* sequence. A 20-bp sequence was chosen from the sequence immediately 5' from the NGG protospacer-associated motif (PAM) sequence. Donor DNA generated 50 bp double-stranded DNA, which was amplified using the primers listed in Table 1. *Saccharomyces cerevisiae* CEN.PK2 was transformed with p42HCas9 and plated on a yeast extract peptone dextrose (YPD) agar plate containing 100 μg/mL nourseothricin (cloNAT). Yeast with p42HCas9 was then transformedusing the pRS42H-*GPD1* and pRS42H-*GPD2* plasmids and donor DNA, and plated on a YPD agar plate containing 100 μg/mL cloNAT and 300 μg/mL hygromycin B for transformation with the pRS42H-*GPD1* and pRS42H-*GPD2* plasmd. All strains were aerobically cultured in 10 mL YPD at 30°C for 24 h.

Table 1. Primers used in this work.

Name	Sequence(5'-3')
pRS42H gpd1.1_F	ATCCTCTTACATCACTG GTTTTAGAGCTAGAAATAGCAAG
pRS42H gpd1.1_R	AGTGATGTAAGAGGATAGC GATCATTTATCTTTCACTGCG
Donor gpd1.1_F	GTCATGTTGATTCACACGTCAGAGCTATCT CCTGTCTAAAATTTATTGGAG
Donor gpd1.1_R	AGTGGGGGAAAGTATGATATGTTATCTTT CTCCAATAAATTTTAGACAGG
pRS42H gpd2.1_F	CCGGTAGGTCTTCCATG GTTTTAGAGCTAGAAATAGCAAG
pRS42H gpd2.1_R	ATGGAAGACCTACCGGAGA GATCATTTATCTTTCACTGCG
Donor gpd2.1_F	GACCTATTGCCATTGTTATTCCGATTAATC TATTGTTCAGCCAATTTATC
Donor gpd2.1_R	CTAGTAGTAGTTGTAGAACTTGTGTATAAT GATAAATTGGCTGAACAATA

2.7. Ethanol fermentation

Ethanol fermentation was performed with 100 mL seaweed hydrolysate in a 250 mL Erlenmeyer flask under semianaerobic condition. After enzymatic saccharification, *G. furcata* hydrolysates were fermented at 30 °C and 150 rpm with *S. cerevisiae* CEN.PK2 as a control strain, *S. cerevisiae* CEN.PK2 Cas9:GPD1 and Cas9:GPD2 as the deletion strain of *S. cerevisiae* CEN.PK2. Samples were collected periodically and stored at − 20 °C to determine their ethanol and residual monosaccharide concentrations. The yeast growth rate was determined using the relationship between dry cell weight (DCW) and optical density at 600 nm (OD_{600}). The ethanol yield coeffcient was defined as follows Eq. (4).

$$Y_{EtOH}(g/g) = \frac{[EtOH]_{\max}}{[Monosaccharides]_{\in i}} \quad \text{Eq. (4)}$$

where Y_{EtOH} is the ethanol yield coeffcient (g/g), $[EtOH]_{max}$ is the maximum ethanol concentration achieved during fermentation and $[Monosaccharides]_{ini}$ is the total initial monosaccharide (glucose+ galactose) concentration at the onset of fermentation (g/L).

2.8. Analytical methods

Monosaccharides, acids, HMF and ethanol concentrations in samples were determined using high-performance liquid chromatography(HPLC, Agilent 1100 Series, Agilent. Inc., Santa Clara, USA) equipped with a

refractive index detector (RID). A Bio-Rad Aminex HPX-87Hcolumn (300.0 × 7.8 mm) was operated at 65°C and the samples were eluted with 5 mM sulfuric acid at a flow rate of 0.6 mL/min. The values are reported as means of triplicate experiments.

3. Results and Discussion

3.1. Composition of *G. furcata*

G. furcata composition was analyzed by the AOAC method and was found to contain 62.6% carbohydrate and fiber, 5.3% moisture, 19.2% crude protein, 0.2% crude lipid and 12.7% crude ash (Table 1.). *G. furcata* has a higher carbohydrate and fiber content than brown and green seaweed, making it a potential substrate for the production of liquid fuels.

Table 2. Composition analysis of *G. furcata* (Feed and Foods Nutrition Research Center, AOAC method).

Species	Seaweed	Composition(%)				
		Fiber	Protein	Lipid	Ash	Carbohydrate
Red Seaweed	*Gloiopeltis furcata*	5.3	19.2	0.2	12.7	62.6

3.2. Optimization of thermal acid hydrolysis

As shown in Fig .1 shows optimization of thermal acid hydrolysis with various condition using *G. fucata*. Fig .1(A) is pretratment result of using various types of acid. Using HCl shown highest efficiency of pretreatment 38%. The optimal acid was shown in Fig. 1(A) and using HCl showed the highest efficiency of pretretment. Fig. 1(B) is result of using various concentrations of HCl. Using 300 mM HCl shown highest efficiency of pretreatment 40.5%. Fig. 1(C) is result of hydrolysis time. And the optimal time was 90 min, efficiency of pretreatment was 38.4%. and Fig. 1(D) is result of slurry contents 6-14%. Optimal slurry contents was 10% , and efficiency of pretreatment was 38%. Therefore the optimal pretreatment conditions of 10% *G. furcata* were 300mM HCl at 121℃ for 90min. After pretreatment Glucose and galactose total concentrations were 26 g/L, respectively.

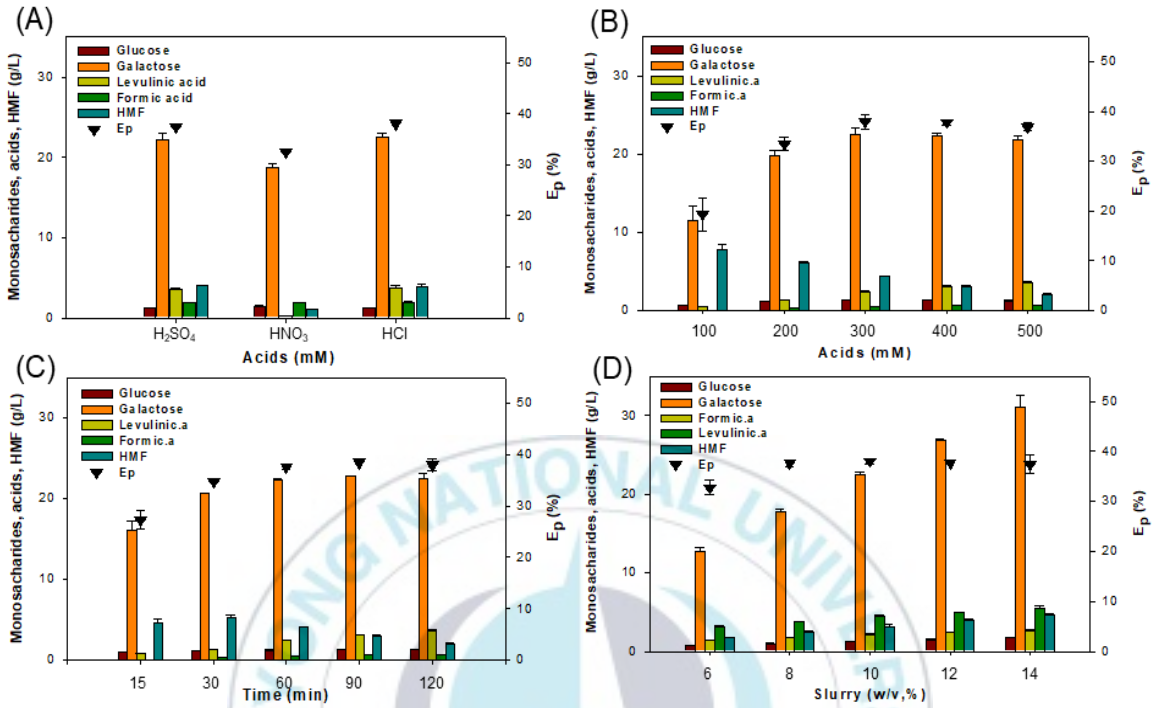

Fig 1. Optimization of thermal acid hydrolysis with various conditions using *G. furcata* (A) various types of acid, (B) concentration of HCl, (C) hydrolysis time and (D) slurry contents.

3.3. Enzymatic saccharification

Glucose and galactose content after enzymatic saccharification of *G. furcata* was determined and is displayed in Fig .2. Viscozyme L , Cellic CTec2 and Celluclast 1.5 L treatments of thermal acid hydrolysate were evaluated for monosaccharides release from 10% (w/v) slurry of *G. furcata* following thermal acid hydrolysis. Effect of enzymatic saccharification with commercial enzymes to reduce more sugar. Using Cellic CTec2 showed the highest efficiency of saccharification about 46.7%. after enzymatic saccharification, glucose and galactose total concentrations were 55.3 g/L.

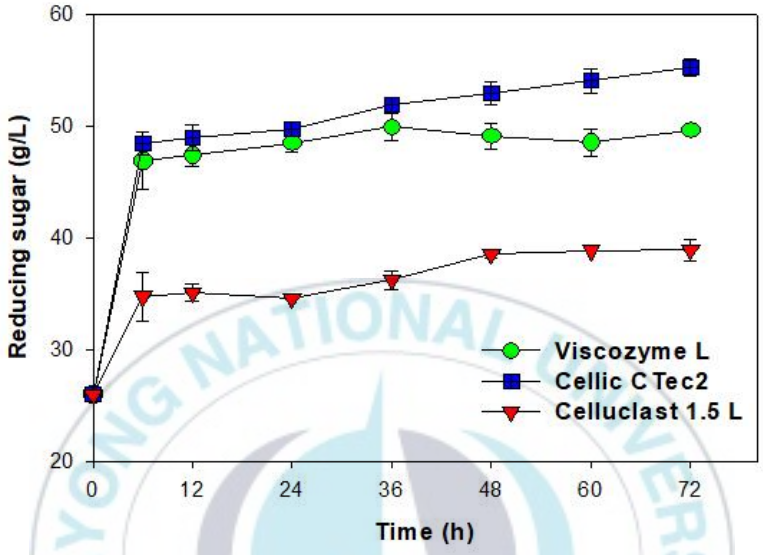

Fig 2. Effect of enzymatic saccharification with commercial enzymes. Enzymatic saccharification of *G. furcata* hydrolysate using Viscozyme L, Cellic CTec2 and Celluclast 1.5L. E_S (Efficiency of saccharification, %).

3.4. HMF removal

HMF, levulinic acid and formic acid are the fermentation inhibitors. However, levulinic acid and formic acid are the weak fermentation inhibitors and over 10 g/L formic acid and 46 g/L levulinic acid showed inhibition of cell growth. According to this study, the hydrolysate contained 1.6 g/L formic acid and 6.3 g/L levulinic acid. Thus, HMF was removed by activated carbon in this study. HMF adsorption with various activated carbon concentrations and adsorption times in 10% (w/v) *G. furcata* hydrolysate was carried out as shown in Fig .3. The HMF removal and monosaccharide concentration for various activated carbon concentrations with the adsorption time of 2 min. The adsorption efficiency depends on the concentration of activated carbon, and monosaccharides can be adsorbed at high activated carbon concentrations. Thus, an appropriate activated carbon concentration was evaluated with the aim of minimizing monosaccharide adsorption. HMF was increasingly removed from the hydrolysate with increasing activated carbon concentrations. The efficiency of HMF removal using activated carbon powder with the adsorption surface areas of 1400 - 1600 m^2/g was higher than granular activated carbon with the adsorption surface areas of 1000 - 1100 m^2/g. Thus, 4% (w/v) activated carbon powder with adsorption time of 2 min were selected to achieve total HMF removal in this study.

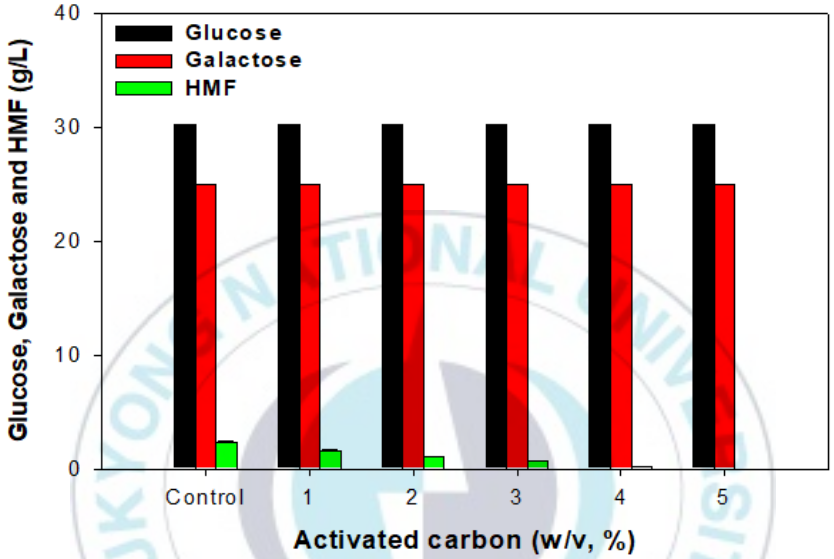

Fig 3. Effect of detoxification using activated carbon (0-5%) on HMF removal for 2 min of adsorption time at 50℃.

3.5. Fermentation

Fig. 4(A) shows ethanol production using *S. cerevisiae* CENpk2-1 wild type. Glucose was completely consumed at 24 h. And during 96 h wild type did not consume galactose. After fermentation, the ethanol production was 20.4 g/L with ethaol Yield 0.37. Fig. 4(B) shows the ethanol production using *S. cerevisiae* CEN.PK2 the deletion of GPD1 gene. Glucose was completely consumed at 24 h. And yeast did not consume galactose well. After fermentation, ethanol production was 20.6 g/L with yield 0.37. It was not much different with wild type so the deletion of GPD1 *S. cerevisiae* it doesn't significantly affect to ethanol production. Fig. 4(C) shows the ethanol production using *S. cerevisiae* CEN.PK2 the deletion of GPD2 gene. Glucose was completely consumed at 24 h and galactose was consumed at 60 h. After fermentation ethanol production was 28.4 g/L with Yield 0.51. Normally *S. cerevisiae* cannot consume galactose but in this work the deletion of GPD2 gene *S. cerevisiae* consumed galactose. More research will find out what the reason is. Fig .4(D) shows the ethanol production using *S. cerevisiae* CEN.PK2 the deletion of both GPD1 and GPD2 gene. Glucose was completely consumed at 36h and galactose did not consume well. After fermentation ethanol production was 19.4 g/L with yield 0.35. The deletion of both two gene, *S. cerevisiae* grow was very slow, and it can be seem that gpd1 gene and GPD2 gene affect the growth of *S. cerevisiae*.

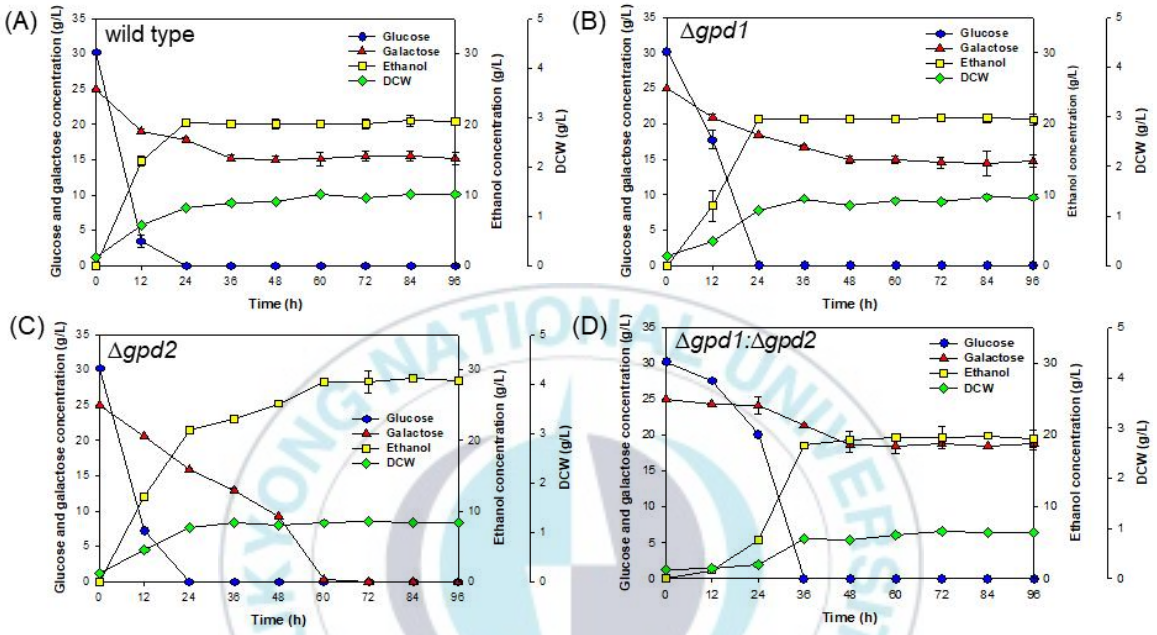

Fig 4. Ethanol production from 10% (w/v) *G. furcata*. *S. cerevisiae* CEN.PK2 wild type (control), (B) *S. cerevisiae* CEN.PK2 pRS42H-Cas9 gpd1, (C) *S. cerevisiae* CEN.PK2 pRS42H-Cas9 gpd2 and (D) *S. cerevisiae* CEN.PK2 pRS42H-Cas9 gpd1:gpd2.

4. Conclusion

Thermal acid hydrolysis of *G. furcata* hydrolysate was successfully optimized using HPLC. The optimal pretreatment conditions using 10% *G. furcata* were 300mM HCl at 121℃ for 90 min. To increase glucose and galactose, the commercial enzyme Cellic CTec2 showed the highest enzymatic saccharification efficiency. After thermal acid hydrolysis and enzymatic saccharification, monosaccharides concentration reached to 44.3 g/L. Fermentation with *S. cerevisiae* CEN.PK2 the deletion of *GPD2* produced 28.4 g/L of ethanol with YEtOH = 0.50 from 10% *G. furcata*.

5. References

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911 – 917.

P. Börjesson, Good or bad bioethanol from a greenhouse gas perspective—Whatdetermines this? Appl. Energy 86 (2009) 589 – 594.

M.D. Torres, S. Kraan, H. Domínguez, Seaweed biorefinery, Rev. Environ. Sci.Biotechnol. 18 (2019) 335 – 388.

R. Jiang, K.N. Ingle, A. Golberg, Macroalgae (seaweed) for liquid transportationbiofuel production: what is next? Algal Res. 14 (2016) 48 – 57.

R. Harun, J.W.S. Yip, S. Thiruvenkadam, W.A. Ghani, T. Cherrington,M.K. Danquah, Algal biomass conversion to bioethanol – a step-by-step assessment,Biotechnol. J. 9 (2014) 73 – 86.

M. Yanagisawa, S. Kawai, K. Murata, Strategies for the production of high concentrations of bioethanol from seaweeds: production of high concentrations ofbioethanol from seaweeds, Bioengineered 4 (2013) 224 – 235.

E.C. Bensah, M. Mensah, Chemical pretreatment methods for the production ofcellulosic ethanol: technologies and innovations, Int. J. Chem. Eng. 2013 (2013)1 – 21.

C.E. Wyman, B. Yang, Combined severity factor for predicting sugar recovery inacid-catalyzed pretreatment followed by enzymatic hydrolysis, in: H.A. Ruiz,M. Thomsen, H.L. Trajano (Eds.),

Hydrothermal Process, Biorefineries, SpringerInternational Publishing, Cham, 2017, pp. 161 – 180.

M. Jeya, N.-P.-T. Nguyen, H.-J. Moon, S.-H. Kim, J.-K. Lee, Conversion of woodybiomass into fermentable sugars by cellulase from Agaricus arvensis, Bioresour.Technol. 101 (2010) 8742 – 8749.

M. Yang, J. Zhang, S. Kuittinen, J. Vepsäläinen, P. Soininen, M. Keinänen,A. Pappinen, Enhanced sugar production from pretreated barley straw by additivexylanase and surfactants in enzymatic hydrolysis for acetone – butanol – ethanol fermentation, Bioresour. Technol. 189 (2015) 131 – 137.

Norbeck, J., Påhlman, A. K., Akhtar, N., Blomberg, A., &Adler, L. (1996). Purification and characterization of two isoenzymes of DL-glycerol-3-phosphatase from *Saccharomyces cerevisiae*: Identification of the corresponding GPP1 and GPP2 genes and evidence for osmotic regulation of Gpp2p expression by the osmosensing mitogen-activated protein kinase signal transduction pathway. Journal of Biological Chemistry, 271(23), 13875-13881.

Påhlman, Anna-Karin, et al. "The yeast glycerol 3-phosphatases Gpp1p and Gpp2p are required for glycerol biosynthesis and differentially involved in the cellular responses to osmotic, anaerobic, and oxidative stress." Journal of Biological Chemistry 276.5 (2001): 3555-3563.

Remize, F., Barnavon, L., &Dequin, S. (2001). Glycerol export and glycerol-3-phosphate dehydrogenase, but not glycerol phosphatase,

are rate limiting for glycerol production in S*accharomyces cerevisiae*. Metabolic engineering, 3(4), 301-312.

Jiang, T., Xiao, D., &Gao, Q. (2008). Characterisation of maltose metabolism in lean dough by lagging and non-lagging baker's yeast strains. Annals of microbiology, 58(4), 655-660.

Cunniff, P. (1995). Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC). AOAC, Virginia,, 2.

Kim, S. R., Xu, H., Lesmana, A., Kuzmanovic, U., Au, M., Florencia, C., ... &Jin, Y. S. (2015). Deletion of PHO13, encoding haloacid dehalogenase type IIA phosphatase, results in upregulation of the pentose phosphate pathway in *Saccharomyces cerevisiae*. Applied and environmental microbiology, 81(5), 1601-1609.

Nguyen TH, Ra CH, Sunwoo IY, Jeong GT, Kim S-K (2016) Evaluation of galactose adapted yeasts for bioethanol fermentation from Kappaphycus alvarezii hydrolyzates. J Microbiol Biotechnol 26:1259 - 126

Kim N-J, Li H, Jung K, Chang HN, Lee PC (2011) Ethanol production from marine algal hydrolysates using *Escherichia coli*KO11. Bioresour Technol 102:7466 - 746922.

Guo Z, Olsson L (2014) Physiological response of *Saccharomyces cerevisiae* to weak acids present in lignocellulosic hydrolysate. FEMS Yeast Res 14:1234 - 124824.

Gütsch JS, Sixta H (2011) The HiTAC-process (high temperature adsorption on activated charcoal)—new possibilities in autohydrolysate treatment. Lenzing Ber 89:142 - 15125.

Zhao X, Moates GK, Elliston A, Wilson DR, Coleman MJ, Waldron KW (2015) Simultaneous saccharification and fermentation of steam exploded duckweed: improvement of the ethanol yield by increasing yeast titre. Bioresour Technol 194:263–26923.

Ra CH, Jeong G-T, Shin MK, Kim S-K (2013) Biotransformation of 5-hydroxymethylfurfural (HMF) by Scheffersomyces stipitisduring ethanol fermentation of hydrolysate of the seaweed Gelidium amansii. Bioresour Technol 140:421–42527.