Bioethanol has attracted attention as an alternative to petroleum-derived fuel. Seaweeds have been proposed as some of the most promising raw materials for bioethanol production because they have several advantages over lignocellulosic biomass. However, because seaweeds contain low contents of glucans, i.e., polysaccharides composed of glucose, the conversion of only the glucans from seaweed is not sufficient to produce high concentrations of ethanol. Therefore, it is also necessary to produce ethanol from other specific carbohydrate components of seaweeds, including sulfated polysaccharides, mannitol, alginate, agar and carrageenan. This review summarizes the current state of research on the production of ethanol from seaweed carbohydrates for which the conversion of carbohydrates to sugars is a key step and makes comparisons with the production of ethanol from lignocellulosic biomass. This review provides valuable information necessary for the production of high concentrations of ethanol from seaweeds.

Introduction

In recent years, bioethanol has attracted attention as an alternative to petroleum-derived fuels. Sugar-rich and starch-based materials are easily converted to ethanol and have therefore been recognized as the most feasible raw materials of bioethanol. However, these materials are also edible crops, so their use in bioethanol production competes with their use as food. Therefore, it is necessary to develop technologies for the efficient production of ethanol from inedible biomass.

Lignocellulosic biomass, such as wood,1-3 rice straw,4 wheat straw,5 switchgrass6 and sugarcane bagasse,7,8 has also been used as the raw material for bioethanol production. Although lignocellulosic biomass is inedible, the presence of lignin makes the cellulose within it resistant to hydrolysis. Therefore, various pretreatment methods, such as steam explosion,1,2,5 organosolv pretreatment,2,6 ionic-liquid pretreatment,2 and ammonia fiber expansion,6 have been applied to accelerate the hydrolysis of cellulose in lignocellulosic biomass. These pretreatments of lignocellulosic biomass increase the energy requirement of the ethanol production process. Furthermore, the cultivation of lignocellulosic biomass may invade arable land, which is required for the production of edible crops.

On the other hand, seaweeds contain low concentrations of lignin9 or no lignin at all.10 Consequently, the conversion of carbohydrates contained in seaweeds into ethanol does not require delignification. Moreover, the cultivation of seaweeds does not invade arable land. Based on these characteristics, seaweeds have been proposed as some of the most promising raw materials for efficient bioethanol production that would not compete with food.

Seaweeds are classified into three groups: green, brown and red. They contain various types of glucans, i.e., polysaccharides composed of glucose. The glucans found in green, brown and red seaweeds are cellulose and starch, cellulose and laminarin and cellulose and floridean starch, respectively.11 Because the hydrolysis of glucans produces glucose, the primary feedstock for ethanol production, the combination of hydrolysis of glucans and fermentation of the resulting glucose allows the production of ethanol from seaweeds. However, the conversion of only the glucans contained in seaweeds into ethanol is not sufficient to produce high concentrations of ethanol, due to the low glucan contents of seaweeds, as described later in this review.

One of the most energy-intensive steps in ethanol production is the recovery of ethanol from fermentation broth by distillation.12 In order to make the process economically feasible, the concentration of generated ethanol should reach 4–5%.13 Therefore, concentrations of higher than 4% (40 g/L ethanol) must be produced from seaweeds in order to develop an economically feasible process for bioethanol production.

The production of ethanol concentrations higher than 40 g/L has not been achieved by converting only seaweed glucans.14,15 The production of high concentrations of ethanol from seaweeds will require the conversion of carbohydrates other than glucan. Green seaweeds contain sulfated polysaccharides, such as ulvan;16 brown seaweeds contain mannitol and alginate;11 and red seaweeds contain agar or carrageenan.17 These carbohydrates represent the major non-glucan carbohydrate contents of seaweed and...
must be converted into ethanol in order to produce high concentrations of ethanol from seaweeds.

This review summarizes the current state of research on the production of ethanol from major carbohydrates contained in seaweeds for which the conversion of carbohydrates to sugars is also the key step and compares seaweed-based ethanol production with the production of ethanol from lignocellulosic biomass. To our knowledge, there have been no previous reviews that comprehensively describe the information required for the production of high concentrations of ethanol from seaweeds.

**Properties of Seaweeds as the Raw Materials for Bioethanol Production**

Several kinds of seaweed are used for industrial applications, including the production of alginate, agar and carrageenan and for foods such as nori, wakame and kombu. Worldwide total industrial consumption and total food use of seaweeds are 1.54 million wet tonnes per year, respectively.\(^1\) Bioethanol could be produced from seaweeds that are not used for these applications. Some of the unused seaweeds may be utilized as feed for shellfish and as habitats for fish in the marine ecosystem, but cultivated seaweeds and seaweeds that are not utilized in the marine ecosystem would be suitable for use as raw materials for bioethanol production.

The production of large amounts of bioethanol from cultivated biomass requires highly productive biomass. Experiments on small-scale cultivation of green seaweeds such as *Ulva*, *Chaetomorpha* and *Cladophora* have suggested that the productivity of volatile solids, corresponding to organic compounds, contained in these seaweeds is around 50 tons volatile solids/(ha·y).\(^1\) Habig et al.\(^2\) have determined that the volatile-solid contents of *Ulva* spp. are approximately 70% on a dry-weight basis. From these values, the productivity of green seaweeds can be calculated to be approximately 7,100 dry g/(m\(^2\)·y). The productivity of brown seaweeds such as *Macrocytis*, *Laminaria*, *Ecklonia* and *Sargassum* ranges from 3,300–11,300 dry g/(m\(^2\)·y) and red seaweeds exhibit a similar range of productivity.\(^3\) Table 1 compares the productivities of several kinds of lignocellulosic biomass used as raw materials for bioethanol production with those of seaweeds. Because the productivities of seaweeds are higher than those of lignocellulosic biomass, seaweeds are promising for the cultivation of biomass used as raw materials for bioethanol production.

| Biomass             | Productivity [dry g/(m\(^2\)·year)] | Reference |
|---------------------|-------------------------------------|-----------|
| Lignocellulosic biomass |                                    |           |
| Switchgrass         | 560–2,240                           | 65        |
| Corn stover         | 180–790                             | 65        |
| Eucalyptus          | 1,000–2,000                         | 65        |
| Poplar              | 300–612.5\(^a\)                    | 66        |
| Willow              | 46–2,700                            | 67        |
| Seaweeds            |                                    |           |
| Green seaweeds      | 7,100\(^b\)                         | 19, 20    |
| Brown seaweeds      | 3,300–11,300                        | 21        |
| Red seaweeds        | 3,300–11,300                        | 21        |

\(^{a}\)Mean value calculated from the amount of biomass produced for 8 y; \(^{b}\)calculated value.

Moreover, seaweed wastes can also be used as raw materials for bioethanol production. The accumulation of green seaweeds causes green tide, a type of harmful algal bloom; the resulting large quantities of accumulated green seaweeds pose a nuisance. In Japan, free-floating *Ulva* accumulates on the shores of seas and lakes. Decayed *Ulva* causes environmental problems, such as killing of shellfish and emission of an offensive odor.\(^2\) In France, up to 98,000 m\(^3\) algal biomass, principally *Ulva*, is gathered during summer along the Breton coastline.\(^2\) It has been speculated that in China, around 20 million wet tonnes of green seaweeds were produced along the shores of Qingdao in 2008.\(^2\) The invasive species *Gracilaria salicornia*, classified as a red seaweed, is commonly found in Hawaii,\(^5\) where large decomposing piles of this seaweed have drawn complaints from residents and driven tourists away.\(^2\) The adequate disposal of these seaweed wastes, which are regarded as a nuisance, probably involves high energies and costs. The production of bioethanol from these seaweed wastes could lead to their effective disposal in addition to their economically advantageous use.

**Production of Ethanol from Glucans Contained in Seaweeds**

Green, brown and red seaweeds contain various types of glucans, as described in the introduction section. Ethanol can therefore be easily produced from seaweeds by hydrolyzing glucans to obtain glucose and fermenting of the resulting glucose. However, the low glucan contents in seaweeds reduce the yields and concentrations of the ethanol produced in this manner. Table 1 compares the glucan contents in various kinds of biomass and the yields and concentrations of ethanol produced from these glucans.\(^1,3,5,15,26\) Glucans listed in Table 2 are represented on a dry-weight basis. Yields and concentrations of ethanol in Table 2 are obtained by combining the enzymatic hydrolysis of glucans with the fermentation of glucose. In the cases of lignocellulosic biomass, pretreatment methods were applied in order to accelerate enzymatic hydrolysis. The glucan contents of seaweeds are lower than those of untreated lignocellulosic biomass. Moreover, the pretreatment of lignocellulosic biomass increases the relative glucan content by removing components other than glucan, such as lignin and hemicellulose. Collectively, the glucan contents of seaweeds are significantly lower than those of pretreated lignocellulosic biomass. As a result, the yields of ethanol from seaweeds are very low compared with those from lignocellulosic biomass. For this reason, the use of pretreated lignocellulosic biomass as raw material enables the production of more than 40 g/L of ethanol, whereas ethanol concentrations produced from seaweed are generally lower than 25.5 g/L. From these data, it can be concluded that the low glucan content and the production of ethanol solely from glucans decrease the yields...
and concentrations of ethanol produced from seaweeds. Although the conversion of glucans alone is not sufficient to produce high concentrations of ethanol from seaweeds at high yields, seaweeds also contain various kinds of major carbohydrates other than glucans, such as sulfated polysaccharides, mannitol, alginate, agar and carrageenan. The conversion of these carbohydrates, along with glucans, into ethanol would increase the yields and concentrations of ethanol produced from seaweeds. The production of sugars and ethanol from green, brown and red seaweeds is described below.

**Production of Sugars and Ethanol from Green Seaweeds**

Green seaweeds contain glucans (i.e., cellulose and starch) and sulfated polysaccharides (e.g., ulvan) as major carbohydrates. Glucans from green seaweeds have been converted to ethanol, but the conversion of sulfated polysaccharides from green seaweeds into ethanol has not yet been performed.

*Ulva pertusa*, classified as a green seaweed, has also been used as the raw material for ethanol production by combining the enzymatic hydrolysis of glucans and the fermentation of glucose. A concentration of 7.2 g/L ethanol was produced from *U. pertusa* by simultaneous saccharification and fermentation at 35°C for 72 h, using crude enzymes containing cellulase and amylase together with *Saccharomyces cerevisiae*. The crude enzymes used to hydrolyze glucans in *U. pertusa* during simultaneous saccharification and fermentation were obtained from the mid-gut gland of scallops, which is ordinarily wasted after the removal of edible parts. Because crude enzymes were used for the hydrolysis of glucans, the conversion rate of glucans from *U. pertusa*, which is defined as the ratio of the amount of ethanol produced by simultaneous saccharification and fermentation to the potential amount of ethanol produced from glucans contained in the seaweed, was not higher than 37%. A commercial enzyme (Meicelase) was also used for the hydrolysis of glucans contained in *U. pertusa*. A concentration of 18.5 g/L ethanol was produced from this seaweed by enzymatic hydrolysis of glucans with Meicelase at 50°C for 120 h and subsequent fermentation of the resulting glucose by *S. cerevisiae*. The yield of ethanol from seaweed using this method for ethanol production was 0.062 g-ethanol/g-seaweed, as shown in the comparison in Table 2. Moreover, the concentration of ethanol was enhanced by applying acid hydrolysis with 2% sulfuric acid at 121°C for 30 min prior to the enzymatic hydrolysis of glucans, resulting in a final concentration of 27.5 g/L ethanol. In this hydrolysis of glucans in *U. pertusa* with acid and enzyme, the concentration of the seaweed was as high as 300 g/L; the conversion rate of glucans in the seaweed, which is defined as the ratio of the amount of glucose produced by hydrolysis to the potential amount of glucose in the seaweed, was 80.6%. Regardless, the concentration of ethanol produced by the fermentation of the hydrolysate did not reach 40 g/L. The yield of ethanol from seaweed in this ethanol production was 0.092 g-ethanol/g-seaweed. In ethanol production from pretreated lignocellulosic biomass, the yields of ethanol were higher than 0.247 g-ethanol/g-pretreated biomass, as summarized in Table 2. An ethanol yield of 0.092 g-glucose/g-seaweed, as calculated above, is not sufficient to produce high concentrations of ethanol.

*Monostroma nitidum*, a green seaweed, has also been used as the raw material for the production of glucose. Glucans in this seaweed were hydrolyzed using a commercial enzyme (Cellulosin T2) at 37°C for 48 h. Although the conversion rate of glucans in the seaweed reached 79.9%, as a result of applying hydrothermal pretreatment at 100°C for 30 min prior to enzymatic hydrolysis, the yield of glucose from the seaweed was still only 0.107 g-glucose/g-seaweed. Even the production of ethanol from aspen (wood) biomass, which with an ethanol yield of 0.247 g-ethanol/g-biomass has the lowest ethanol yield from pretreated lignocellulosic biomass (summarized in Table 2), requires the hydrolysis of glucans with glucose yields higher than 0.5 g-glucose/g-biomass. This glucose yield from biomass was calculated by assuming that the ethanol yield from glucose is

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### Table 2. Glucan contents in various kinds of biomass used for bioethanol production and the yields and concentrations of ethanol produced from glucans in the biomass

| Biomass                  | Glucan content in untreated biomass [%]| Glucan content in pretreated biomass [%]| Yield of ethanol from pretreated biomass (g-ethanol/g-pretreated biomass) | Concentration of ethanol (g/L) | Reference |
|--------------------------|----------------------------------------|-----------------------------------------|--------------------------------------------------------------------------------|-------------------------------|-----------|
| Lignocellulosic biomass  |                                        |                                         |                                                                                |                               |           |
| Wood (Japanese cedar)    | 43.5<sup>a</sup>                        | 89.1<sup>c-d</sup>                     | 0.367<sup>d</sup>                                                              | 73.3                          | 2         |
| Wood (aspen)             | 45.6                                    | 66.2                                    | 0.285<sup>d</sup>                                                              | 60                            | 3         |
| Wood (aspen)             | 47.7                                    | 50.3                                    | 0.247<sup>b</sup>                                                              | 47                            | 1         |
| Wheat straw              | 31.5<sup>a</sup>                        | 67.2<sup>c</sup>                       | 0.308<sup>d</sup>                                                              | 51.5                          | 5         |
| Corn stover              | 39.5<sup>a</sup>                        | 69.7<sup>c</sup>                       | 0.308<sup>d</sup>                                                              | 52.3                          | 26        |
| Seaweeds                 |                                        |                                         |                                                                                |                               |           |
| Green seaweed (*Ulva pertusa*) | 22                                    | -                                       | 0.062<sup>a</sup>                                                            | 18.5                          | 15        |
| Brown seaweed (*Alaria crassifolia*) | 24.5                                 | -                                       | 0.085<sup>a</sup>                                                            | 25.5                          | 15        |
| Red seaweed (*Gelidium elegans*) | 21.8                                 | -                                       | 0.061<sup>a</sup>                                                            | 18.4                          | 15        |

<sup>a</sup>Enzymatic hydrolysis was used for the hydrolysis of glucans; <sup>b</sup>dry-weight basis; <sup>c</sup>described as cellulose; <sup>d</sup>calculated values. <sup>e</sup>yield of ethanol from untreated biomass.
0.5 g-ethanol/g-glucose and dividing the ethanol yield from biomass (0.247 g-ethanol/g-biomass) by the ethanol yield from glucose (0.5 g-ethanol/g-glucose). The resulting glucose yield of 0.107 g-glucose/g-seaweed from the hydrolysis of M. nitidum is too low. In general, in the conversion of glucans in green seaweeds into glucose and ethanol, the yields of glucose and ethanol did not reach sufficiently high values to produce a concentration of 40 g/L ethanol. Therefore, in order to produce high concentrations of ethanol from green seaweeds, carbohydrates other than glucans must be converted to ethanol. Recently, the production of acetone, butanol and ethanol (ABE) from biomass of the green seaweed Ulva lactuca has been reported. In this study, over 90% of sugars (rhamnose, galactose, glucose and xylose) was solubilized by hot-water treatment followed by hydrolysis using commercial cellulase cocktail (GC220, Genencor) and Clostridium beijerincki produced ABE at high yields (0.35 g ABE/g sugar consumed) from the hydrolysate.

Green seaweeds contain sulfated polysaccharides as major non-glucan carbohydrates. Ulvan is a water-soluble sulfated polysaccharide found in Ulva and Enteromorpha sp. that mainly consists of repeating disaccharide units composed of sulfated rhamnose and glucuronic acid, iduronic acid and xylose or sulfated xylose (Fig. 1). For example, ulvans from Ulva fasciata are mainly composed of rhamnose, xylose and glucuronic acid, whereas those from Ulva armoricana are mainly composed of rhamnose, glucuronic acid and iduronic acid. The sulfated groups of these sugars in ulvans are likely to be removed by acid hydrolysis during determinations of sugar compositions in ulvans, because acid treatment releases all sulfated groups from ulvans. Ulvan contents of green seaweeds are 8–29% on a dry-weight basis. A variety of other forms of sulfated polysaccharides are synthesized by green seaweeds such as Enteromorpha compressa and M. nitidum.

Although ethanol has not been produced from sulfated polysaccharides contained in green seaweeds, the hydrolysis of these polysaccharides has been performed to produce sugars. Furthermore, each of the resulting components, such as xylose and glucuronic acid, has been converted into ethanol. This field has also been reviewed by Weber et al. With regard to the hydrolysis of sulfated polysaccharides, Feng et al. attempted to hydrolyze Enteromorpha with dilute sulfuric acid at 121°C for 30–90 min and obtained a mixture of glucose, xylose, glucuronic acid and rhamnose. The sulfated groups of these sugars seemed to be removed by acid hydrolysis, as described above. The yield of glucose alone from the seaweed was 0.1752 g-glucose/g-seaweed, similar to the yield obtained from enzymatic hydrolysis of M. nitidum, whereas the yield of total sugar including glucose, xylose, glucuronic acid and rhamnose was as high as 0.5 g-sugar/g-seaweed. Therefore, if ethanol could be efficiently produced from these sugars (glucose, xylose, glucuronic acid and rhamnose), it would be possible to produce ethanol from this seaweed at high yields. Conversion into ethanol has been attempted for two of these sugars, xylose and glucuronic acid, as described in the following paragraphs.

To convert xylose to ethanol, a xylose-fermenting yeast,39 a xylose-utilizing recombinant of S. cerevisiae,37 and an ethanologenic recombinant of Escherichia coli38 were used. Xylose-utilizing S. cerevisiae and ethanologenic E. coli produced ethanol from xylose with ethanol yields higher than 0.4 g-ethanol/g-xylose.

On the other hand, the production of ethanol from glucuronic acid has been attempted using Pachysolen tannophilus35 and ethanologenic E. coli.36 P. tannophilus indeed produced ethanol from glucuronic acid, but the yield and production rate are unclear due to the lack of information about the amounts of glucuronic acid consumed and ethanol produced during the fermentation. From ethanologenic E. coli, the yield of ethanol from glucuronic acid was approximately 0.2 g-ethanol/g-glucuronic acid. Moreover, it would be worth noting that genes for bacterial D-galacturonic acid isomerase (uxaC) and bacterial D-tagaturonic acid reductase (uxaB) have been successfully expressed in S. cerevisiae. D-galacturonic acid is a component of pectin, a third major carbohydrate polymer in plant and this study would be the first step to realize pectin fermentation by S. cerevisiae.

Production of Sugars and Ethanol from Brown Seaweeds

Brown seaweeds contain glucans (i.e., cellulose and laminarin), mannitol and alginate as major carbohydrates. Several hydrolysis
methods have been applied to brown seaweeds in order to produce sugars. Furthermore, the fermentation of sugars produced by the hydrolysis of brown seaweeds has been conducted. In addition, the major carbohydrates contained in brown seaweeds have been converted to ethanol without hydrolysis.

Acid, enzymatic and hydrothermal hydrolysis methods have been applied to brown seaweeds. Yeon et al.42 performed hydrothermal hydrolysis of *Sargassum sagamianum* at 200°C for 15 min. Lee et al.43 applied acid hydrolysis using 5% (v/v) sulfuric acid at 120°C for 24 h to *Undaria pinnatifida*. These hydrolysis methods produced several sugars, including glucose, xylose, fructose and mannose, but required high energies because the treatments were conducted at an extremely high temperature or for a very long time.

On the other hand, enzymatic hydrolysis has been used to produce glucose under milder conditions from glucans contained in *Laminaria japonica* ("makombu" in Japanese),10,44 *U. pinnatifida* ("chum"").14,45 *Sargassum fulvellum*,44 and *Alaria crassifolia* ("chigaiso").15 Although acid pretreatment or acid hydrolysis was applied prior to enzymatic hydrolysis in some cases, those treatments were conducted using dilute acid, such as 0.1% (w/v) sulfuric acid or 0.1 N hydrochloric acid, under relatively mild conditions, e.g., 121°C for up to 1.5 h.10,44

The yields and concentrations of sugars produced by the hydrolysis of brown seaweeds are compared in Table 3.10,14,15,43,44 The maximum reported yield of glucose produced from brown seaweeds is 0.2775 g-glucose/g-pretreated seaweed from the enzymatic hydrolysis of acid-pretreated *L. japonica*. This yield is higher than that of glucose from green seaweeds and indicates that hydrolysis at a concentration of 290 g/L pretreated seaweed can produce approximately 80 g/L of glucose, which is calculated as the product of the glucose yield of 0.2775 g-glucose/g-pretreated seaweed and a pretreated seaweed concentration of 290 g/L (80 = 0.2775 × 290). The fermentation of 80 g/L glucose to ethanol with high yield would enable the production of 40 g/L ethanol; however, the concentration of glucose produced from the pretreated seaweed was calculated to be only 5.55 g/L, as shown in Table 3.10

Ethanol production from glucose obtained by the enzymatic hydrolysis of brown seaweeds has also been attempted.10,15 In these experiments, *L. japonica*10 and *A. crassifolia*15 were used as the raw materials for ethanol production. In the production of ethanol from *L. japonica*, the seaweed was hydrolyzed at 50°C for 48 h using commercial enzymes (cellulase and cellobiase) following pretreatment of the seaweed with 0.1% (w/v) sulfuric acid at 121°C for 1 h.10 After the enzymatic hydrolysis, glucose in the hydrolysate was concentrated by rotary evaporation, which increased the concentration of glucose in the hydrolysate from 5.55 g/L to 34.0 g/L. A concentration of 14.0 g/L ethanol was produced by the fermentation of the concentrated hydrolysate using *S. cerevisiae*.10 The ethanol yield was 0.114 g-ethanol/g-glucose produced by the fermentation of the concentrated hydrolysate.

Table 3. Yields and concentrations of sugars produced by hydrolysis of brown seaweeds

| Seaweed               | Hydrolysis       | Conditions                  | Sugars produced | Concentration of sugar (g/L) | Reference |
|-----------------------|------------------|-----------------------------|-----------------|------------------------------|-----------|
| *Undaria pinnatifida* | Acid              | 120°C for 24 h              | Glucose         | 0.065                        | 3.3°      | 43        |
|                       | Enzymatic        | 45°C for 60 min             | Glucose         | 0.014                        | 0.7°      | 43        |
| *Laminaria japonica*  | Enzymatic + acid | 50°C for 48 h               | Glucose         | 0.2775                       | 5.55°     | 10        |
|                       | Acid and enzymatic | 50°C for 24 h         | Glucose         | 0.0698                       | 6.98°     | 44        |
| *Sargassum fulvellum* | Acid and enzymatic | 50°C for 24 h          | Glucose         | 0.0596                       | 5.96°     | 44        |
| *Undaria pinnatifida* | Enzymatic + removal of alginate | 50°C for 24 h          | Glucose         | 0.13                          | 0.130°    | 14        |
| *Alaria crassifolia*  | Enzymatic        | 50°C for 120 h             | Glucose         | 0.224°                       | 67.2      | 15        |

°Sulfuric acid was used at 5% (v/v); °°Celluclast 1.5 L and Novozyme 188 were used as enzymes; °commercial cellulase and cellobiase were used as enzymes; °°sulfuric acid was used for the acid pretreatment at a concentration of 0.1% (w/v); °acid-insoluble residue was washed and used for the enzymatic hydrolysis; °hydrochloric acid was used at a concentration of 0.1 N; °°Viscozyme L and Celluclast 1.5 L were used as enzymes; °°°commercial cellulase was used as the enzyme; °°°sodium carbonate was used for the removal of alginate at a concentration of 1% (w/v); °°°°treatment time for the removal of alginate is not described; °°°°°Meicelase was used as the enzyme; °°°°°°yield of ethanol from pretreated seaweed; °°°°°°°°calculated values.
because a high concentration of the seaweed (300 g/L) was used in the hydrolysis; however, the concentration of ethanol still did not reach 40 g/L.\textsuperscript{15}

Although the conversion of only glucose to ethanol is not sufficient to produce high concentrations of ethanol from brown seaweeds, the hydrolysates of brown seaweeds also contain mannitol, which is a soluble sugar alcohol, in addition to glucose.\textsuperscript{14} As shown in Table 3, the yield of total sugar produced by the hydrolysis of \textit{L. japonica} was 0.3752 g-sugar/g-seaweed, which is the sum of a glucose yield of 0.0698 g-glucose/g-seaweed and a mannitol yield of 0.3054 g-mannitol/g-seaweed. This sugar yield is higher than those of other brown seaweeds. Thus, the conversion of mannitol to ethanol probably contributes to the production of high concentrations of ethanol from brown seaweeds.

Mannitol derived from brown seaweeds has been used as the substrate for the production of ethanol. Horn et al.\textsuperscript{45} attempted to extract mannitol from \textit{Laminaria hyperborea} and convert the extracted mannitol into ethanol. Laminarin was also present in this extract. Ethanol was produced from mannitol and/or laminarin using several microorganisms including \textit{Zymobacter palmae}, \textit{Pachysolen tannophilus}, \textit{Kluyveromyces marxianus} and \textit{Pichia angophorae}. \textit{Z. palmae} fermented only mannitol to ethanol, whereas \textit{P. tannophilus} and \textit{K. marxianus} fermented only laminarin and \textit{P. angophorae} fermented both mannitol and laminarin. The highest ethanol concentration achieved in these fermentation methods was approximately 10 g/L.\textsuperscript{45} Kim et al.\textsuperscript{44} applied simultaneous saccharification and fermentation to an acid hydrolysate of \textit{L. japonica} using a mixture of commercial enzymes (Celluclast 1.5L, Novoprimse B959, Novoprimse B969 and Viscozyme L) and ethanologenic microorganisms. The concentrations of ethanol reached approximately 7 and 29 g/L when using \textit{S. cerevisiae} and \textit{E. coli} KO11, respectively, as ethanologenic microorganisms. The authors stated that the difference in the concentrations of ethanol was likely due to the inability of the yeast to utilize mannitol. In the fermentation with \textit{E. coli} KO11, the yield of ethanol from the seaweed was calculated to be approximately 0.161 g-ethanol/g-seaweed. This value is significantly higher than the yields of ethanol from seaweeds shown in Table 2 (lower than 0.085 g-ethanol/g-seaweed) and indicates that an increase in the concentration of the seaweed up to 250 g/L can produce an approximate concentration of 40 g/L ethanol, which is the product of an ethanol yield of 0.161 g-ethanol/g-seaweed and a seaweed concentration of 250 g/L (40 = 0.161 x 250).

The production of ethanol from mannitol in addition to glucose is effective in increasing the concentrations and yields of ethanol produced from brown seaweeds, but not all brown seaweeds have high mannitol contents. In fact, the yield of mannitol from \textit{S. fulvum} after hydrolysis with acid and enzymes was only 0.0215 g-mannitol/g-seaweed, markedly lower than that from \textit{L. japonica} (0.3054 g-mannitol/g-seaweed), as shown in Table 3.\textsuperscript{44} For this reason, the conversion of major carbohydrates other than glucans and mannitol into ethanol is necessary in order to produce ethanol with high yields from various kinds of brown seaweeds, particular in the case of brown seaweeds with low mannitol contents.

Brown seaweeds also contain carbohydrates other than glucans and mannitol, e.g., alginate and fucoidan. Alginate is a linear polyuronic acid consisting of mannuronic acid and guluronic acid. The structure of alginate consists of three types of blocks: the M-block, consisting of mannuronic acid residues; the G-block, consisting of guluronic acid residues; and the MG-block, consisting of alternating mannuronic acid and guluronic acid residues.\textsuperscript{46,47} The structures of these three blocks are shown in Figure 2. Fucoidan is a sulfated fucan that consists of fucose, uronic acid, galactose, xylose and sulfated fucose.\textsuperscript{48} Alginate is the major polysaccharide of brown seaweeds;\textsuperscript{22} therefore, in order to produce ethanol with high yields from various kinds of brown seaweeds, it will probably be necessary to convert alginate to ethanol. However, it is difficult to produce ethanol from alginate, because ethanologenic microorganisms do not utilize alginate or the degradation products of alginate as substrates.\textsuperscript{50} Therefore, there had been no previous studies on the production of ethanol from alginate.

In 2011, our group succeeded in producing ethanol from alginate using \textit{Sphingomonas} sp A1 (strain A1), an alginate-assimilating bacterium, expressing both pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH).\textsuperscript{50} In this first report on the production of ethanol from alginate, the genes encoding the ethanol-producing PDC (\textit{pdc}) and ADH (\textit{adhB}) from \textit{Zymomonas mobilis} were introduced into strain A1. The ethanologenic strain A1 constructed by this method produced ethanol directly from sodium alginate, a commercially available reagent. Moreover, the ethanologenic strain A1 was further modified in order to improve its ethanol productivity. First, DNA microarray analysis revealed the promoter of a gene that is constitutively and highly expressed in strain A1; accordingly, the original promoters of \textit{pdc} and \textit{adhB} were replaced with this promoter to enhance the activities of the corresponding proteins. Second, the copy number of \textit{pdc} was

![Figure 2. The structures of the M-block, G-block and MG-block of alginate.\textsuperscript{47}](image-url)
increased from one to eight copies to further enhance the activity of PDC. Finally, metabolome analysis indicated that lactate is accumulated as the main byproduct in the ethanol production, so the lactate dehydrogenase gene was disrupted, thereby increasing the concentration of ethanol produced from sodium alginate. The modified ethanologenic strain A1 incorporates alginate into the cell and converts it to ethanol by a pathway shown in Figure 3. Although these modifications made it possible to consume 52 g/L of sodium alginate and produce 13 g/L of ethanol after 77 h of fermentation,50 the yield of ethanol from sodium alginate was only 0.25 g-ethanol/g-sodium alginate.

In another study, E. coli was genetically engineered to assimilate alginate and produce ethanol.51 This engineered E. coli strain (BAL1611) converted alginate from L. japonica (described in that paper as Saccharina japonica) into ethanol. In this study, an alginate-lyase secretion system including a DNA fragment encoding alginate lyase from Pseudoalteromonas sp was constructed. In addition, a DNA fragment from Vibrio splendidus was used to promote alginate degradation, transport and metabolism. The construct for the secretion of alginate lyase and the DNA fragment encoding enzymes for the assimilation of alginate were introduced into E. coli along with pdc and adhB from Z. mobilis. Due also to the intrinsic ability of E. coli to metabolize glucose and mannitol, the resulting engineered strain could produce ethanol from various carbohydrates such as alginate, mannitol and glucose from L. japonica; a concentration of 37.8 g/L ethanol was achieved.50 The yield of ethanol from this seaweed was 0.291 g-ethanol/g-seaweed, which is similar to the yields of ethanol from pretreated lignocellulosic biomass, shown in Table 2. It should be noted, however, that the mannitol content of L. japonica, used as the raw material in this study, was extremely high, approximately 50%,51 whereas the mannitol content of S. fulvellum is very low, as shown in Table 3.

Mannitol contents of brown seaweeds seem to vary considerably, depending on the species. For example, the mannitol content of L. japonica is up to 50%, as described above, whereas that of S. fulvellum is only 2.15%, as shown in Table 3. However, alginate constitutes as much as 14–40% of the dry solids of brown seaweeds.11 In particular, the alginate content in blades of U. pinnatifida collected in April is up to 51%.52 Therefore, in order to produce high concentrations of ethanol from brown seaweed, it will be necessary to develop processes specific to each species and variety.

Production of Sugars and Ethanol from Red Seaweeds

Red seaweeds contain agar or carrageenan as major carbohydrates, in addition to glucans such as cellulose and floridean starch. Agar consists of agarose and agarapectin.53 Agarose is a polysaccharide that consists of repeating disaccharide units composed of β-d-galactose and 3,6-anhydro-α-d-galactose (Fig. 4; R1 = R2 = R3 = H). The replacement of some hydroxyl groups in agarose by various functional groups results in agarapectin (Fig. 4).53,54 Carrageenan is a generic name for a family of polysaccharides that includes six basic forms: iota-, kappa-, lambda-, mu-, nu- and theta-carrageenans.55 Mu-, nu- and lambda-carrageenans mainly consist of repeating disaccharide units composed of β-d-galactose and α-d-galactose, whereas kappa-, iota- and theta-carrageenans mainly consist of repeating disaccharide units composed of β-d-galactose and 3,6-anhydro-α-d-galactose. These repeating disaccharide units contain some sulfate groups (Fig. 5).

Agar and carrageenans are polysaccharides that contain galactose, as shown in Figures 4 and 5. Common ethanologenic microorganisms, such as S. cerevisiae, possess the ability to ferment galactose into ethanol. Thus, high concentrations of total fermentable sugar, i.e., the sum of the concentrations of glucose and galactose, are present in red seaweeds. The efficient conversion of fermentable sugars to ethanol would probably enable increased yields of ethanol from red seaweeds.

Gelidium amansii56,57 and Kappaphycus alvarezii58-61 have been used as the raw materials for acid hydrolysis to produce sugars.
K. alvarezii and general Gelidium spp. contain the galactose-containing polysaccharides kappa-carrageenan and agar, respectively. The yields and concentrations of sugars produced by acid hydrolysis of red seaweeds are compared in Table 4. In many cases, both glucose and galactose were produced from red seaweeds. The concentrations of total fermentable sugar, i.e., the sum of the concentrations of glucose and galactose, were lower than 35 g/L in all cases summarized in Table 4. These concentrations of total fermentable sugar would not be sufficient to produce a concentration of 40 g/L ethanol. In fact, the concentrations of ethanol produced from the acid hydrolysates without first concentrating fermentable sugars were lower than 6.8 g/L. In order to produce higher concentrations of ethanol, sugars in the acid hydrolysate of G. amansii and K. alvarezii were concentrated. Fermentable sugars in the acid hydrolysate of G. amansii were concentrated by evaporation, and the concentrated hydrolysate was used as the substrate for ethanol production, resulting in a final ethanol concentration of 27.6 g/L. By contrast, reducing sugars in the acid hydrolysate of K. alvarezii were concentrated by another method. First, hydrolysis of seaweed was conducted in 0.9 N sulfuric acid and hydrolysate was filtered and used again for a second hydrolysis. In the second hydrolysis, new seaweed was hydrolyzed in the filtrate of the hydrolysate prepared by the first hydrolysis. This process was repeated a total of five times and 24.1 g/L of ethanol was produced from the fifth hydrolysate. The concentrations of ethanol produced from the acid hydrolysates of red seaweeds did not reach 40 g/L due to the low concentrations of total fermentable sugar. For this reason, high concentrations of total fermentable sugar must be achieved in order to produce high concentration of ethanol from red seaweeds. The highest yield of total fermentable sugar from acid hydrolysis of red seaweeds was 0.35 g-fermentable sugar/g-seaweed, which is the sum of a glucose yield of 0.05 g-glucose/g-seaweed and a galactose yield of 0.30 g-galactose/g-seaweed, as shown in Table 4. This yield of total fermentable sugar indicates that acid hydrolysis at a concentration of 230 g/L seaweed can produce approximately 80 g/L of glucose, which is the product of a total fermentable sugar yield of 0.35 g-fermentable sugar/g-seaweed and a seaweed concentration of 230 g/L (80 = 0.35 × 230). However, an increase in the concentration of seaweeds in the acid hydrolysis did not allow the production of higher concentrations of fermentable sugars. In addition, dilute-acid hydrolysis appears to be ineffective for hydrolysis of glucans, because the yields and concentrations of glucose produced by this method were extremely low, as shown in Table 4. Therefore, an increase in the yields and concentrations of glucose, resulting from application of a hydrolysis method other than dilute-acid hydrolysis, will be necessary in order to produce high concentrations of fermentable sugars from red seaweeds.

Enzymatic hydrolysis has also been applied to hydrolyze glucans contained in red seaweeds. High yields of total fermentable sugar were obtained by combining acid hydrolysis and enzymatic hydrolysis. Sugar mixtures containing glucose and galactose as major fermentable sugars were obtained from G. amansii and Gelidium elegans, with yields of 0.565 g-sugar/g-seaweed and 0.414 g-sugar/g-seaweed, respectively. In addition, the concentration of glucose produced by enzymatic hydrolysis could be increased by increasing the concentration of seaweed. In fact, a fermentable sugar mixture containing 70.9 g/L of glucose and 53.2 g/L of galactose was produced from a concentration of 300 g/L G. elegans by applying acid hydrolysis with 2% sulfuric acid at 121°C for 30 min and subsequent enzymatic hydrolysis with Meicelase at 50°C for 120 h. Furthermore, this fermentable sugar mixture was converted to a concentration of up to 55.0 g/L ethanol, which corresponds to an ethanol yield of 0.183 g-ethanol/g-seaweed. This study succeeded in producing a high concentration of ethanol (55.0 g/L), although the yield of ethanol was lower than those from pretreated lignocellulosic biomass, shown in Table 2 (higher than 0.247 g-ethanol/g-pretreated biomass). Moreover, 3,6-anhydrogalactose contained in the seaweeds was not converted to ethanol. Since seaweeds of Gelidium spp contain agar, the G. elegans used in this study probably contains...
3,6-anhydro-α-L-galactose as a component of agar. Therefore, converting 3,6-anhydrogalactose to ethanol might increase the yield of ethanol from red seaweeds and thereby contribute to the efficient production of ethanol from red seaweeds.

However, no information is available regarding the fermentability and metabolism of 3,6-anhydrogalactose. Furthermore, the amounts of 3,6-anhydrogalactose produced by the acid hydrolysis of red seaweeds decreased with increasing hydrolysis time or acid concentration. It appears that 3,6-anhydrogalactose is easily degraded by acid. The efficient production of ethanol from 3,6-anhydrogalactose included in agar or carrageenans of red seaweeds requires both the hydrolysis of these polysaccharides to obtain 3,6-anhydrogalactose with high conversion rates and the fermentation of 3,6-anhydrogalactose into ethanol with high yields.

**Conclusion and Perspective**

The lignins contents of seaweeds are very low; therefore, the carbohydrates contained in seaweeds should be easily converted to ethanol. However, because the glucan contents of seaweeds are low, the conversion of only the glucans contained in seaweeds is not sufficient to produce high concentrations of ethanol. Thus, the production of high concentrations of ethanol from seaweeds requires the conversion of every major carbohydrate into ethanol. The highest concentrations of ethanol produced from green, brown and red seaweeds are summarized in Table 5. The polysaccharides, sugars in them and organisms to convert these sugars into ethanol were summarized in Table 6.

A concentration of 55.0 g/L ethanol was produced from *G. elegans*, classified as a red seaweed. Although the ethanol yield from this seaweed is lower than that from *L. japonica*, as compared in Table 5, a high concentration of seaweed, i.e., 300 g/L, in the hydrolysis allowed production of the highest reported concentration of ethanol from seaweed. In that study, 3,6-anhydrogalactose contained in the seaweed was not converted to ethanol. Therefore, the conversion of 3,6-anhydrogalactose in addition to glucose and galactose into ethanol with high conversion rates will further increase the concentrations and yields of ethanol produced from brown seaweeds containing low concentrations of mannitol.

In order to hydrolyze polysaccharides other than glucans, acid hydrolysis has been applied to seaweeds. However, acid hydrolysis at high temperatures, e.g., higher than 100°C, increases the energy requirement. Additionally, acid hydrolysis causes the decomposition of monosaccharides into undesirable compounds, such as furfural and hydroxymethylfurfural, which are known to decompose 3,6-anhydrogalactose in the fermentation. Therefore, the conversion of 3,6-anhydrogalactose in addition to glucose and galactose into ethanol with high conversion rates will further increase the concentrations and yields of ethanol produced from red seaweeds.

### Table 5. Yields and concentrations of sugars produced by acid hydrolysis of red seaweeds

| Seaweed         | Conditions of acid hydrolysis | Sugars produced | Yield of sugar [g-sugar/g-seaweed] | Concentration of sugar [g/L] | Reference |
|-----------------|--------------------------------|-----------------|-----------------------------------|------------------------------|-----------|
| *Gelidium amansii* | 1% H₂SO₄ at 150°C for 15 min  | Glucose         | ca. 0.05d                         | ca. 5                        | 57        |
| *Gelidium amansii* | 2% H₂SO₄, 150°C for 4 h        | Glucose         | ca. 0.05d                         | ca. 5                        | 56        |
|                  |                                 | Galactose       | ca. 0.25d                         | ca. 25                       |           |
| *Kappaphycus alvarezii* | H₂SO₄, 130°C for 15 min         | Glucose         | <c                                | 0.89                         | 60        |
| *Kappaphycus alvarezii* | 0.2 M H₂SO₄, 150°C for 15 min  | Glucose         | 0.0078d                           | 0.78                         | 68        |
|                  |                                 | Galactose       | 0.2239d                           | 22.39                        |           |
| *Kappaphycus alvarezii* | 0.2 M H₂SO₄, 130°C for 15 min  | Glucose         | 0.0089d                           | 0.89                         | 59        |
|                  |                                 | Galactose       | 0.2387d                           | 23.87                        |           |

*The concentration of sulfuric acid used in this study is unclear; \(^{3,6}\)-anhydro-L-galactose; it is impossible to calculate the yields of sugars in this study, because the concentration of seaweed for hydrolysis is unclear; \(^{a}\)calculated values.*
to inhibit subsequent fermentation.64 In contrast to acid hydrolysis, enzymatic hydrolysis is conducted at low temperatures, e.g., lower than 50°C. Moreover, enzymatic hydrolysis does not cause the decomposition of monosaccharides. Therefore, enzymes for the hydrolysis of polysaccharides other than glucans with high conversion rates may be required for the efficient production of ethanol from seaweeds. Furthermore, the use of purified enzymes tends to be associated with high costs. Therefore, in order to produce bioethanol from seaweeds in a cost-effective manner, microorganisms that possess the ability to directly convert polysaccharides (including glucans) into ethanol must be screened or constructed.

Disclosure of Potential Conflicts of Interest
The authors declare no competing financial interests.

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Table 5. The highest concentrations of ethanol produced from green, brown and red seaweeds

| Seaweed              | Carbohydrates converted to ethanol | Ethanol concentration [g/L] | Ethanol yield [g-ethanol/g-seaweed] | Reference |
|----------------------|-----------------------------------|-----------------------------|-------------------------------------|-----------|
| Ulva pertusa         | Glucans                           | 27.5                        | 0.092b                              | 15        |
| (Green seaweed)      |                                    |                             |                                     |           |
| Laminaria japonica*  | Glucose                           | 37.8                        | 0.291b                              | 51        |
| (Brown seaweed)      | Mannitol                          |                             |                                     |           |
| Gelidium elegans     | Glucans                           | 55                          | 0.183b                              | 15        |
| (Red seaweed)        | Agar (galactose)                  |                             |                                     |           |

*Described in that study as Saccharina japonica; b calculated values.

Table 6. Polysaccharides, sugars in them and organisms to convert these sugars into ethanol

| Biomass         | Polysaccharides | Sugar          | Organism                  | Reference |
|-----------------|-----------------|----------------|---------------------------|-----------|
| Green seaweed   | Glucan          | Glucose        | S. cerevisiae             | 15, 27    |
|                 | Ulvan           | Xylose         | Xylose-fermenting yeast   | 39        |
|                 |                 |                | Ethanologenic E. coli     | 38        |
|                 | Glucuronic acid |                | P. tannophilus            | 35        |
|                 |                 |                | Ethanologenic E. coli     | 36        |
| Brown seaweed   | Glucan          | Glucose        | S. cerevisiae             | 10, 15    |
|                 |                 |                | Ethanologenic E. coli KO11| 44        |
|                 |                 |                | Ethanologenic E. coli BAL1611| 51        |
|                 | Mannitol        |                | Ethanologenic E. coli KO11| 44        |
|                 |                 |                | Ethanologenic E. coli BAL1611| 51        |
|                 | Alginate        | Uronic acid    | Ethanologenic Sphingomonas sp. A1| 50        |
|                 |                 |                | Ethanologenic E. coli BAL1611| 51        |
| Red seaweed     | Glucan          | Glucose        | S. cerevisiae             | 15, 56, 58, 60, 61 |
|                 | Agar, Carrageenan| Galactose      | S. cerevisiae             | 15, 56, 58, 60, 61 |
|                 | 3,6-anhydrogalactose|            | NRa                       |           |

*Mannitol is not a polysaccharides, but a major sugars in brown seaweeds; a ethanol production from 3,6-anhydrogalactose has not been reported.
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