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Ethanol production from seaweed extract†

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Extracts from *Laminaria hyperborea* could possibly be fermented to ethanol commercially. In particular, seaweed harvested in the autumn contains high levels of easily extractable laminaran and mannitol. Four microorganisms were tested to carry out this fermentation, one bacterium and three yeasts. Only *Pichia angophorae* was able to utilise both laminaran and mannitol for ethanol production, and its substrate preferences were investigated in batch and continuous cultures. Laminaran and mannitol were consumed simultaneously, but with different relative rates. In batch fermentations, mannitol was the preferred substrate. Its share of the total laminaran and mannitol consumption rate increased with oxygen transfer rate (OTR) and pH. In continuous fermentations, laminaran was the preferred substrate at low OTR, whereas at higher OTR, laminaran and mannitol were consumed at similar rates. Optimisation of ethanol yield required a low OTR, and the best yield of 0.43 g ethanol (g substrate)$^{-1}$ was achieved in batch culture at pH 4.5 and 5.8 mmol O_2 l^{-1} h^{-1}. However, industrial production of ethanol from seaweed would require an optimisation of the extraction process to yield a higher ethanol concentration. *Journal of Industrial Microbiology & Biotechnology* (2000) **25,** 249–254.

Keywords: *Pichia angophorae*; *Zymobacter palmae*; *Laminaria hyperborea*; mannitol; laminaran; biofuel

Introduction

Brown seaweeds may have a high content of easily degradable carbohydrates, making them a potential substrate for the production of liquid fuels. In autumn fronds of *Laminaria hyperborea* from the Norwegian coast, 25% and 30% of the dry weight have been reported to be mannitol and laminaran, respectively [4]. Most of the work concerning bioconversion of seaweed has been related to methane gas production. Production of liquid fuels such as ethanol has received very little attention, probably because alginate, the main constituent of brown seaweed, cannot be utilised directly as a substrate for ethanol production. Laminaran and mannitol, on the other hand, may be converted to ethanol, and these sugars can also easily be extracted from milled seaweed [9].

Laminaran is a linear polysaccharide of (1→3)-β-D-glucopyranose, with chains terminated by D-mannitol. Low levels of branching by (1→6)-β-glucosidic linkages do occur [7]. β-(1→3)-Glucanases are relatively widespread, and many microorganisms can hydrolyse laminaran to its glucose monomer, a good substrate for fermentation. Mannitol, on the other hand, is not readily fermented. It is initially oxidised to fructose by the enzyme mannitol dehydrogenase, a reaction that generates NADH. Regeneration of NAD^+ requires oxygen or transhydrogenase, which converts NADH to NADPH. Thus, many microorganisms are not able to carry out strictly anaerobic fermentation of mannitol [11].

Today the most important microorganisms used for ethanol production are *Saccharomyces cerevisiae* and *Zymomonas mobilis*. However, both these ethanol producers have a very narrow substrate range. Yeast lacks transhydrogenase [11] and experiments done with *Saccharomyces* led to the conclusion that pure anaerobic growth on mannitol was not possible [10]. Thus, fermentation of sugar alcohols by yeast requires a supply of oxygen. The bacterium *Zymobacter palmae* has a broad substrate range and can convert mannitol to ethanol [8]. In a previous work, we showed that *Z. palmae* can produce ethanol from mannitol in seaweed extract if a supply of oxygen is provided. Laminaran, however, was not utilised, indicating a lack of β-(1→3)-glucanase [2].

A successful utilisation of seaweed extract for ethanol production necessitates that both mannitol and laminaran are converted to ethanol at high yields. This may be achieved by two different organisms in a two-step process each optimised for maximum ethanol yield. Ethanol production may also be carried out by a single organism that can utilise both substrates. In this case, an effective single-step process may be achieved only if both substrates can be converted to ethanol with high yields under similar conditions. In this work the possibility of ethanol production from *L. hyperborea* extracts was evaluated, focusing on the yeast *Pichia angophorae* and its potential of utilizing both mannitol and laminaran for ethanol production.

Materials and methods

Microorganisms

Four different microorganisms were used: one bacterium, *Z. palmae* T109 (ATCC 51623) and three yeast strains: *P. angophorae* (CBS 5830), *Kluyveromyces marxianus* (NCYC 1426) and *Pacchysolen tannophilus* (NCYC 614). The cultures were maintained at −80°C in 15% glycerol.

Medium

The defined cultivation medium consisted of (g l^{-1}): mannitol, 40.0; yeast extract (Oxoid L21), 0.3; malt extract (Oxoid L39), 0.3; $(NH_4)_2SO_4$, 5.0; KH_2PO_4, 2.0; and $MgSO_4 \cdot 7H_2O$, 0.4.

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The seaweed medium was prepared by extraction of fresh autumn fronds of *L. hyperborea*. One kilogram of milled (7-mm sieve) wet weight fronds was extracted in 1 l tap water at pH 2.0 and 65°C [9] for 1 h, yielding extracts with approximately 20 g l^{-1} of both mannitol and laminaran. The extractions were carried out in 3-l fermentors (Applicon, Schiedam, Netherlands). The seaweed particles were removed by filtering the suspension through a nylon cloth with a mesh size of 90 μm, and the final extracts were stored at −20°C. The pH was adjusted to about 4.5 with 4 g concentrated NH_4OH solution (25%) per kilogram extract before autoclaving (121°C, 20 min) the extract.

Culture conditions and experiments

Experiments in shake flasks were carried out in 250-ml Erlenmeyer flasks, where the pH in the seaweed medium was adjusted to 5.0 (7.0 for *Z. palmae*) before autoclaving it. The flasks were inoculated with a pellet of a preculture grown on the defined mannitol medium, giving an initial biomass concentration of about 12 g l^{-1}. The biomass in *Z. palmae* cultures was twice as high. The flasks were incubated on a reciprocal shaker at 30°C.

All other experiments were carried out in 3-l fermentors (Applicon, Schiedam, Netherlands) with 0.75 l working medium. pH was automatically controlled at pH 4.5 by addition of 3 M NaOH (pH 6.0 for the *Z. palmae* culture) and temperature was maintained at 30°C. CO_2 in the outlet gas (Binos 100.2 M gas analyser), stirrer speed and dissolved oxygen were recorded automatically. The fermentors were inoculated with 40 ml of a preculture grown aerobically on mannitol to a cell density of about 0.7 g l^{-1}. The continuous reactor was considered to be in steady state after a period of three times the hydraulic retention time.

Determination of $K_L a$

The oxygen transfer coefficient, $K_L a$, was determined by measuring the oxygen concentration (Rosemount, Oxynos 100 gas analyser, Hanau, Germany) in the outlet airflow of an active stationary phase culture. Defining OUR as the oxygen uptake rate and OTR as the oxygen transfer rate, under steady state conditions $OUR = OTR = K_L a \times (C_S - C)$, where C_S is the dissolved oxygen (DO) concentration in air-saturated medium, and C is the actual concentration in the medium during the fermentation.

Sampling and analytical methods

Samples were taken aseptically, centrifuged (9000×*g*, 3 min), and the supernatants were stored frozen at −20°C until analysis.

Laminaran was generally quantified as total sugars by the phenol–sulphuric acid method [1]. This may result in a biased overestimate, since the method will include other sugars as well. However, mannitol is not detected by this method. Laminaran was also quantified by complete hydrolysis and determination of the glucose concentration by the glucose oxidase method [6]. These values were on average 4.7 g l^{-1} lower than the total sugars method, the difference representing other less biodegradable sugars.

Mannitol, ethanol and organic acids were quantified by high-performance liquid chromatography (HPLC) of samples filtered through a 0.22-μm pore size Millipore filter (Type GS). The analyses were conducted with a Shimadzu LC.9A liquid chromatograph equipped with a Shimadzu column oven CTO-6A, a Shimadzu autoinjector SIL-6A, a Shimadzu refractive index detector RID-6A, a Shimadzu C-R4A integrator, Biorad Aminex HPX-87H column (300×7.8 mm) and Biorad Micro-Guard Cation H^+ refill cartridge guard column. Other conditions included: effluent, filtered and degassed 50 mm H_2SO_4; flow rate, 0.6 ml min^{-1}; and column temperature, 45°C. Calibration of the method against reference compounds was done within a standard error of ±1.5%.

Cell dry weight was quantified by centrifugation (25 ml broth, 3500×*g*, 10 min), washing the cells in distilled water and heating the suspension to constant weight under IR lamps.

Results and discussion

A selection of yeast strains reported to consume mannitol *aerobically*, which should thus possess mannitol dehydrogenase, were tested for their ability to ferment laminaran to ethanol. The three most promising strains, *P. angophorae*, *K. marxianus* and *P. tannophilus*, were tested for fermentation of seaweed extract in shake flasks. *P. angophorae* was reported to produce ethanol from mannitol [5]. Figure 1 shows the fermentation pattern of these three yeasts together with the bacterium *Z. palmae*. The substrate specificity of the different strains is clear. The bacterium *Z. palmae* readily fermented mannitol to ethanol, but was not able to utilise laminaran since it lacks laminarase activity [2]. The yeasts *P. tannophilus* and *K. marxianus*, on the other hand, could utilise laminaran but not mannitol. *P. angophorae* fermented both laminaran and mannitol simultaneously to ethanol. However, the initial consumption rate of laminaran was about half of the rates of *P. tannophilus* and *K. marxianus*. Ethanol yield was in the range 0.29–0.31 g (g substrate)$^{-1}$ for all the yeast strains. Because the oxygen supply is vital for the mannitol turnover, a separate experiment was done with the same three yeast strains, where the working volume was reduced to improve the oxygen supply. Only *P. angophorae* showed a significant improvement of growth rate and ethanol yield (results not included), mainly because the mannitol turnover increased. The other two yeasts were still not able to consume mannitol, and the smaller volume did not affect the laminaran consumption. Thus, *P. angophorae* can consume mannitol under oxygen-limiting conditions, whereas *P. tannophilus* and *K. marxianus* seem to require fully aerobic conditions. Figure 1B and C show that after a quick initial consumption of laminaran, the consumption rate was significantly reduced when approximately 10 g l^{-1} total sugars were left in the medium, corresponding to 5.3 g l^{-1} laminaran when corrected as described in the Materials and methods section. The main polysaccharide chain in laminaran is hydrolysed by β-(1→3)-glucanase. However, the branching occurs *via* (1→6)-β-glucosidic linkages, which cannot be hydrolysed by β-(1→3)-glucanase. Thus, an incomplete hydrolysis would be expected if the yeasts have only enzymes for hydrolysis of (1→3)-β-glucosidic linkages.

Because *P. angophorae* was the only organism that utilised both substrates, further work concentrated on this strain. Figure 2A shows results of an experiment carried out in a fermentor using a defined medium with mannitol as the only carbon source. The growing biomass made the oxygen concentration drop and the culture was oxygen limited from 5 h. The initial specific growth rate, before oxygen limitation occurred, was 0.41 h^{-1}. In the period 16–75 h the cell mass increased about three times, whereas the CO_2 emission rate (CER) was very stable, increasing slowly from 3.5 up to 4.5 mmol l^{-1} h^{-1}. The total yield was 0.40 g ethanol (g mannitol)$^{-1}$. The

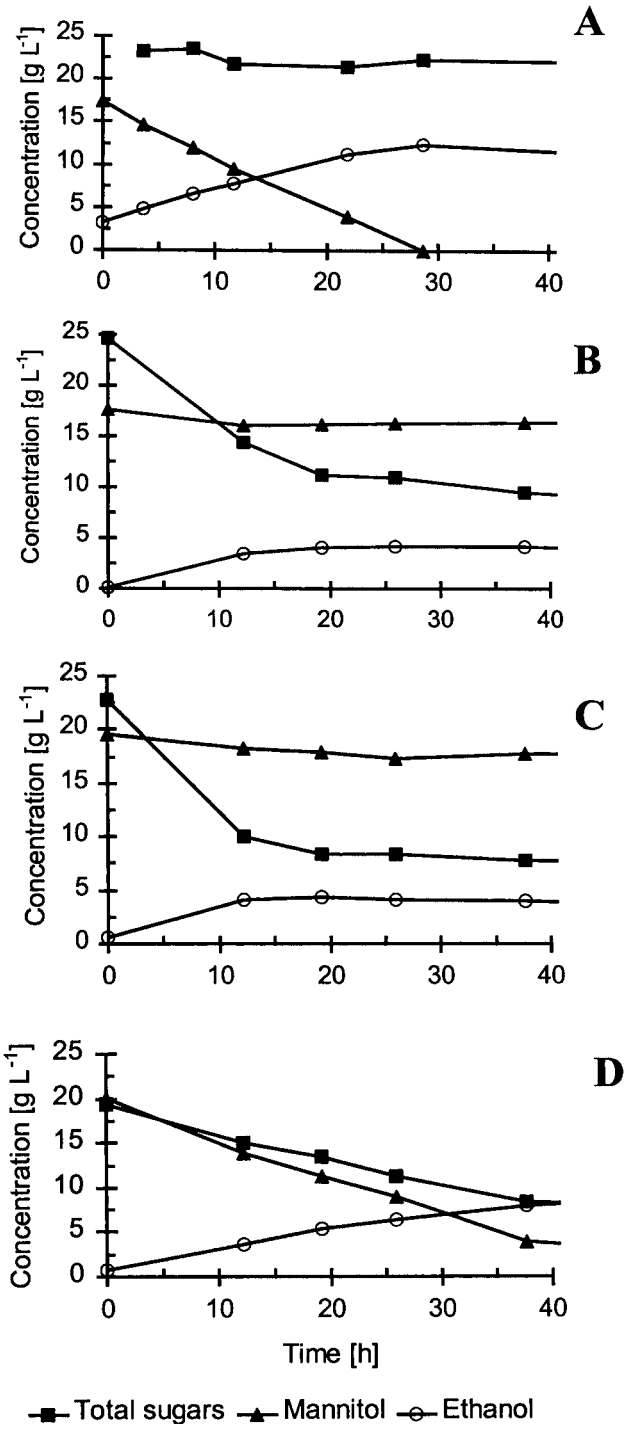

Figure 1 Metabolism of laminaran and/or mannitol in seaweed extract by four different microorganisms: (A) *Z. palmae*, (B) *P. tannophilus*, (C) *K. marxianus* and (D) *P. angophorae*. The initial ethanol in (A) was introduced with the inoculum.

same ethanol yield and a similar fermentation pattern have been observed for *Z. palmae* under similar conditions [2]. However, *P. angophorae* reached a much higher cell mass and had an initial specific ethanol productivity of 0.22 g (g cell h)$^{-1}$, which was more than three times lower than productivity of *Z. palmae*.

A range of experiments was carried out using fermentors to test the influence of pH and OTR on the relative consumption of mannitol and laminaran by *P. angophorae* in seaweed extract. The data from one batch fermentor are shown in Figure 2B. Measurements of optical density or cell dry weight were not possible due to high particle content (about 2 g l^{-1} solids) in the seaweed extract. Cell mass estimates by protein analysis were not quantitative due to interference of the sugars present. Both substrates were consumed simultaneously, but whereas the consumption rate of mannitol increased with the growing biomass, laminaran consumption rate remained constant for a while, and was then strongly reduced at the end of the fermentation. The maximum ethanol concentration and maximum CER coincided with the exhaustion of mannitol. Total yield was 0.26 g ethanol (g substrate)$^{-1}$. In the following period, CER was low and laminaran was consumed slowly together with ethanol. The reason for this late decrease in laminaran consumption rate was probably that *P. angophorae* was unable to hydrolyse branching points due to a lack of β-(1→6)-glucanase, as discussed above. The fermentor culture was oxygen-limited from 12 h until exhaustion of mannitol. Then the oxygen concentration increased rapidly, interrupted by a short, but typical, drop of oxygen around 24 h. The initiation of ethanol consumption could be due to higher oxygen levels and a transfer to

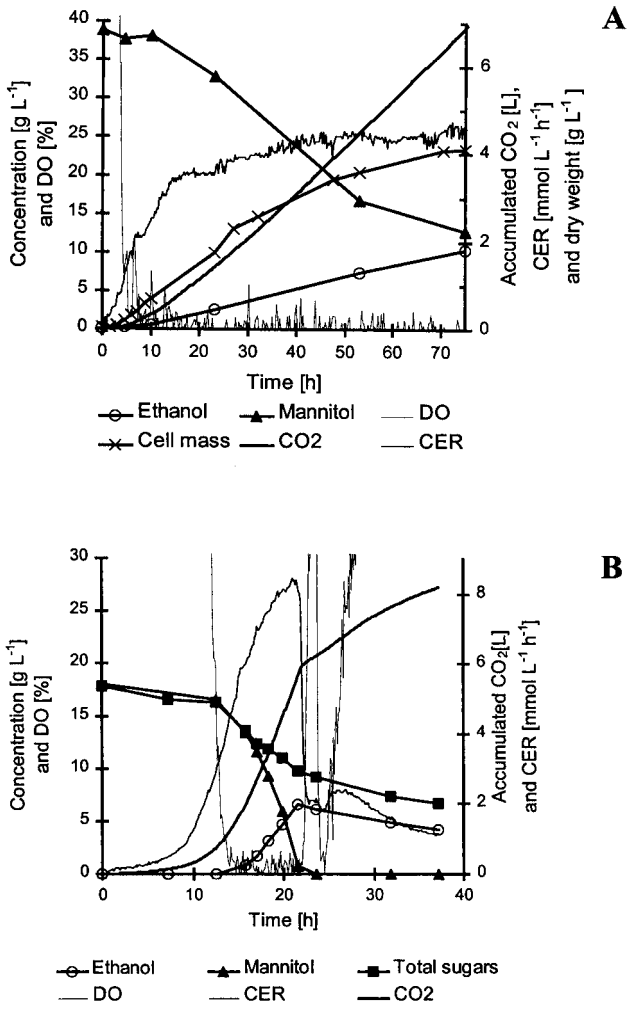

Figure 2 Fermentations with *P. angophorae* at pH 4.5: (A) defined mannitol medium with OTR = 4.7 mmol l^{-1} h^{-1}; (B) seaweed extract medium with OTR = 15.4 mmol l^{-1} h^{-1}. CER = CO_2 emission rate.

Table 1 Operating constants and kinetic parameters OTR=oxygen transfer rate, CER=CO_2 emission rate, *dS*=substrate consumption, *dP*=ethanol production, Pa=*P. angophorae*, Zp=*Z. palmae*, sea=seaweed extract, synt=synthetic mannitol medium, M=mannitol, L=laminaran

Fermentation	pH	OTR [mmol O_2 l^{-1} h^{-1}]	Max. CER[a] [mmol CO_2 l^{-1} h^{-1}]	Ethanol yield [g g^{-1}]	dS/dt [g l^{-1} h^{-1}]	Max. dM/dt[b] [g l^{-1} h^{-1}]	Max. dL/dt[c] [g l^{-1} h^{-1}]	dP/dt [g l^{-1} h^{-1}]
Pa synt	4.5	4.7	4.7 (75.0)	0.40	0.35	0.54		0.14
Pa sea	4.5	5.9	9.7 (23.0)[c]	0.43	1.00	1.26	0.84	0.36
Pa sea	4.5	10.1	30.8 (23.1)	0.23	1.10	2.25	0.80	0.26
Pa sea	4.5	15.4	28.0 (21.2)	0.26	1.20	2.62	0.85	0.31
Pa sea	4.5	16.5	26.5 (20.4)	0.25	1.10	2.51	0.89	0.27
Pa sea	4.5	17.5	26.7 (19.7)	0.26	1.10	2.40	0.80	0.29
Pa sea	4.0	15.4	23.3 (23.1)	0.20	0.90	2.03	0.81	0.18
Pa sea	5.0	15.4	28.1 (18.4)	0.27	1.10	2.78	0.90	0.31
Pa sea	5.5	15.4	25.9 (15.5)	0.18	1.10	2.75	0.92	0.19
Zp/Pa sea[d]	6.0	4.9	5.5 (7.6)	0.18	0.94			0.17
			8.7 (40.9)	0.20	0.85			0.14

[a]Numbers in brackets indicate hours after inoculation.
[b]Average calculated for the interval of 3 succeeding sample points.
[c]In this case max. CER did not coincide with the exhaustion of mannitol. Mannitol was depleted after 28.2 h at a CER of 9.2 mmol CO_2 l^{-1} h^{-1}.
[d]Two growth periods: 0–11 h for Zp and 24–50.5 h for Pa.

aerobic metabolism. The fermentation characteristics observed in the other fermentors were similar, except for the fermentor aerated with 5.9 mmol O_2 l^{-1} h^{-1} (Table 1). Instead of reaching a CER peak at exhaustion of mannitol, CER was stable from around 15 h to exhaustion of mannitol at 28.2 h. This was similar to the experiment in synthetic medium, also carried out at low OTR.

The effect of different OTRs on substrate consumption is shown in Figure 3. Higher OTRs led to faster consumption of mannitol and therefore shorter periods to reach mannitol depletion (Table 1). The consumption rate of laminaran stayed constant in a wide OTR range, but decreased at the two highest OTRs. The total substrate consumption rates, however, were very similar and were not significantly affected by OTR (Figure 3). The share of laminaran consumption compared to the total substrate consumption gradually decreased from 36.5% at 5.9 mmol O_2 l^{-1} h^{-1} to 24.1% at 17.5 mmol O_2 l^{-1} h^{-1}. The decrease was most pronounced above 15.4 mmol O_2 l^{-1} h^{-1}. The maximum mannitol consumption rate at the lowest OTR was 1.3 g l^{-1} h^{-1}, whereas for the other OTRs it was stable in the range 2.3–2.6 g l^{-1} h^{-1} (Table 1). The maximum laminaran consumption rate was stable for all the OTRs ranging from 0.8 to 0.9 g l^{-1} h^{-1}. Laminaran consumption was initiated simultaneously with mannitol consumption, showing that pre-growth in mannitol medium did not repress the laminarase. The laminaran consumption was not directly affected by the oxygen supply. However, indirectly, laminaran consumption decreased with higher OTRs, because of the preference for mannitol as substrate at high OTR. To gain a high ethanol yield it is important to have a low OTR. In the range 10.1 to 17.5 mmol O_2 l^{-1} h^{-1} the

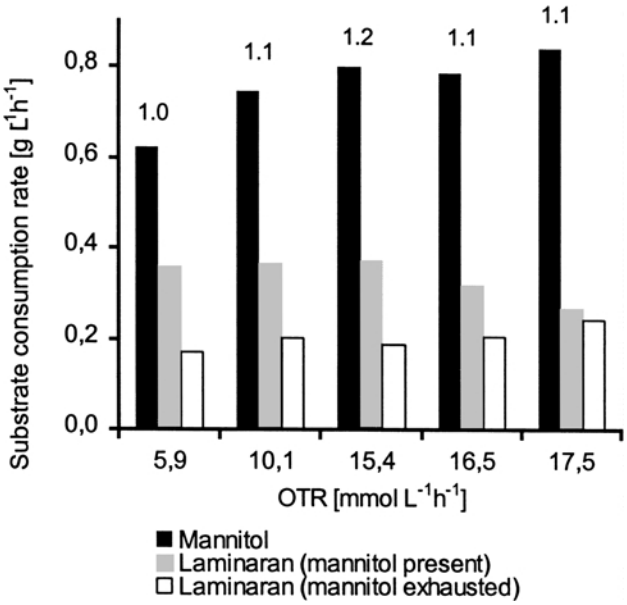

Figure 3 Average consumption rates of substrate in batch cultures of *P. angophorae* at different OTRs. Rates for laminaran consumption were calculated in the periods before and after mannitol exhaustion. The numbers indicate total substrate consumption rates in the first period.

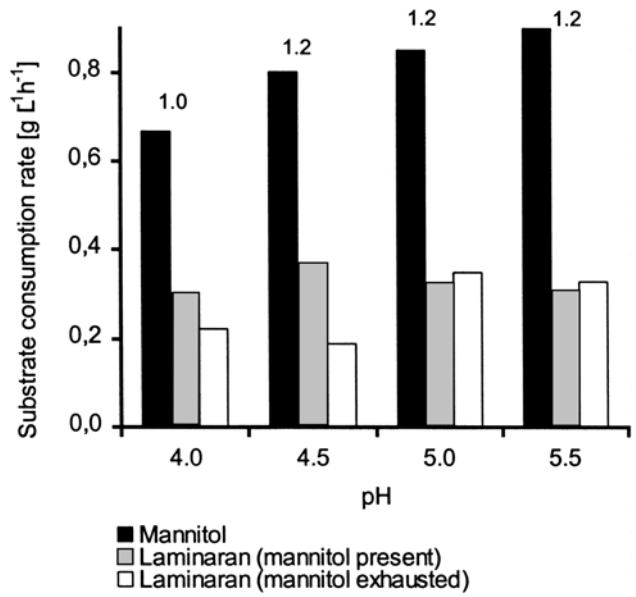

Figure 4 Average consumption rates of substrate at OTR = 15.4 mmol l^{-1} h^{-1} in batch cultures of *P. angophorae* at different pH values. Rates for laminaran consumption were calculated in the periods before and after mannitol exhaustion. The numbers indicate total substrate consumption rates in the first period.

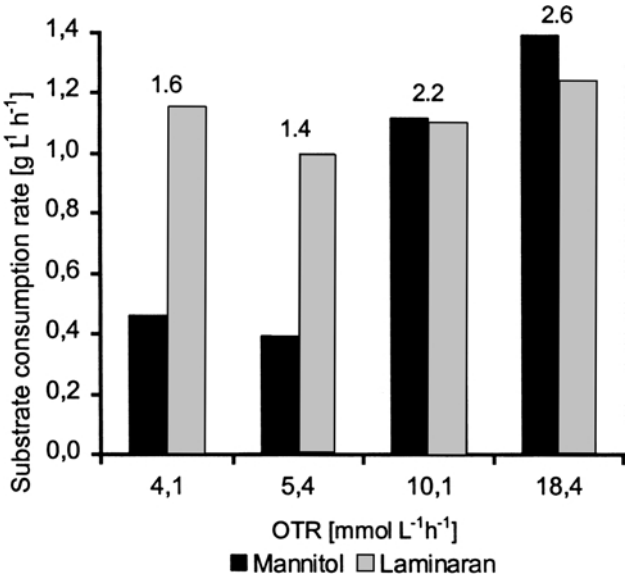

Figure 5 Steady-state consumption rates of substrate in a continuous reactor with *P. angophorae* at different OTRs. The dilution rate was 0.10 h^{-1}. The numbers indicate total substrate consumption rate.

ethanol yield was from 0.23 to 0.26 g ethanol (g substrate)$^{-1}$, whereas the two fermentations at lowest OTR achieved a yield of 0.40 and 0.43 g^{-1} (Table 1). Thus, higher OTR led to a preference for mannitol, but reduced the ethanol yield. After mannitol was exhausted, a decrease in laminaran consumption rate was observed. The reason was probably that the remaining laminaran contained high levels of branching points. In this period, from mannitol depletion until 37 h, laminaran consumption rate was constant at the lowest OTRs, but increased at the two highest. High OTR led to a preference for mannitol consumption, and more main chain laminaran may have remained nonhydrolysed until mannitol was depleted.

The effects of pH are depicted in Figure 4. The consumption rate of mannitol increased with higher pH. At pH 4.0 the maximum rate was 2.0 g l^{-1} h^{-1}, whereas it was in the range 2.6 to 2.8 g l^{-1} h^{-1} for the higher pHs. The maximum laminaran consumption rate was less affected by pH, spanning the range 0.8 to 0.9 g l^{-1} h^{-1}. After mannitol was exhausted, the two highest pH values showed a positive effect on laminaran consumption. This could be due to more main chain laminaran remaining nonhydrolysed through the period with mannitol present, but both fermentors also had end concentrations of 5.3 and 5.9 g l^{-1} total sugars, the lowest concentrations achieved in any batch experiment. Table 1 shows that to achieve maximum ethanol yield, pH should be kept between 4.5 and 5.0. However, the optimisation of ethanol yield requires a lower OTR than was used in these pH experiments.

The substrate specificity was somewhat different in a continuous culture: although mannitol was the preferred substrate in batch cultures, laminaran consumption rate was now greater or similar to the consumption rate of mannitol (Figure 5). The system was run at a dilution rate of 0.10 h^{-1}, that is, one-fourth of the maximum growth rate in defined medium. The seaweed extract fed to the continuous reactor contained 24.1 g l^{-1} mannitol and 20.7 g l^{-1} total sugars (that is, about 7 g l^{-1} more carbohydrate than the extract used in the batch experiments). The continuous medium feed resulted in steady-state concentrations of total sugars in the range 6.5 to 8.5 g l^{-1} for all OTRs, the lower value probably representing sugars inaccessible to degradation. Thus, the continuous culture experienced laminaran limitation. Mannitol concentrations were in the range 18–20 g l^{-1} for the three lowest OTRs, decreasing to 10 g l^{-1} at 18.4 mmol h^{-1} l^{-1}. This shows that complete utilization of mannitol would require a very high OTR at this dilution rate. Ethanol yield, on the other hand, decreased with higher OTR, with a maximum of 0.27 g ethanol (g substrate)$^{-1}$ at 4.1 mmol h^{-1} l^{-1}. Dissolved oxygen concentration was maintained at zero in the whole OTR range.

The maximum consumption rates for laminaran in the batch experiments were in the range 0.8–0.9 g l^{-1} h^{-1}, which is somewhat lower than the continuous rates. However, the maximum mannitol consumption rates in batch were much higher than in continuous cultures, probably due to a higher cell concentration at the end of the batch fermentations. This higher cell mass did not result in enhanced laminaran consumption in the batch reactors, since laminaran at this stage probably was enriched in high levels of branching points. The cell mass was not quantified, but for different fermentations with a similar CER to OTR ratio, CER should be proportional to the cell mass. In the continuous fermentor at 4.1 and 5.4 mmol O_2 l^{-1} h^{-1}, the average CER was 3.8 and 5.0 mmol CO_2 l^{-1} h^{-1}, respectively. For the batch fermentation at 5.9 mmol O_2 l^{-1} h^{-1}, a maximum CER of 9.7 mmol CO_2 l^{-1} h^{-1} was reached, indicating a higher cell biomass. Also, more than twice as much substrate was consumed in this batch fermentation. Thus, the lower mannitol consumption rate in continuous systems at low OTR was probably caused by a lower cell mass concentration, compared to batch experiments. It was only at the highest OTR of 18.4 mmol O_2 l^{-1} h^{-1} that the continuous system had a CER similar to the batch fermentations (26 mmol CO_2 l^{-1} h^{-1}) (Table 1). The limiting factors for the cell mass concentration were OTR and hydraulic retention time (10 h). Thus, the solution for improving the utilization of mannitol while maintaining a high ethanol yield would be to increase the retention time, or maintain a higher cell biomass in the reactor by biomass retention.

Figure 6 shows the data for a culture of *Z. palmae* grown in seaweed extract. As reported earlier [2], the activity of *Z. palmae* ceased before mannitol was exhausted, probably because of lack of some growth factor. However, when *P. angophorae* was added after 24 h to this nonactive culture, both substrates started to be consumed. This shows that *P. angophorae* is a more suitable organism for ethanol production from seaweed extract. It can utilise

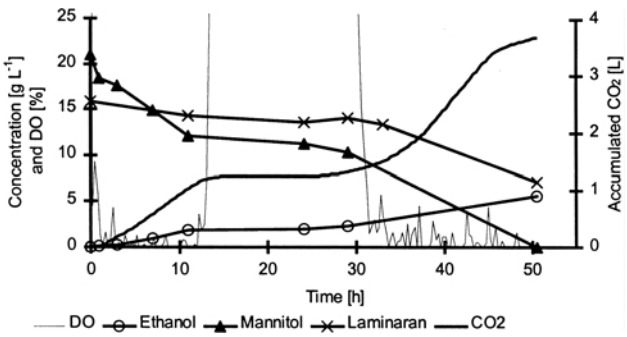

Figure 6 Fermentation with *Z. palmae* in seaweed extract with OTR = 4.9 mmol l^{-1} h^{-1}. At 24 h the fermentor was inoculated with *P. angophorae* (10 ml from the culture aerated with 5.9 mmol O_2 l^{-1} h^{-1}, which was running simultaneously with the *Z. palmae* culture). Note that laminaran here was measured directly with the glucose oxidase method.

both substrates, and is not inhibited before the substrate is consumed.

Utilisation of seaweed carbohydrates for ethanol production is probably only of economic interest when integrated with a balanced and total utilization of the seaweed material. The largest organic fraction of *L. hyperborea* is alginate, which is exploited on an industrial scale for alginate production. In Norway alone, the annual production is 6400 tonnes of alginate, isolated from about 150 000 tonnes of fresh weight *L. hyperborea* [3]. In this extraction process, mannitol and laminaran are washed out and disposed into the sea, representing an organic load for the recipient. In such a case, seaweed extract with mannitol and laminaran may be considered to be non-cost material for ethanol production. The excess organic residues in such a process could be fermented to methane.

This work was not concerned with the final ethanol concentration, which certainly would be a critical factor in industrial production of ethanol. Running a distillation on a low ethanol concentration will not be economical. In our laboratory, extractions carried out with less water than described above gave up to 30 g l^{-1} of both laminaran and mannitol. This would yield a maximum ethanol concentration of no more than 3%. Thus, optimisation of the extraction process is necessary to obtain higher ethanol concentrations. Access to dried seaweed material may also serve this purpose.

Conclusions

P. angophorae was shown to ferment mannitol and laminaran in seaweed extracts simultaneously to ethanol. In batch experiments, mannitol was the preferred substrate, and higher OTR and pH favoured mannitol consumption. However, optimisation of ethanol yield required a low OTR and a pH between 4.5 and 5.0. Maximum yield was 0.43 g ethanol (g substrate)$^{-1}$ at pH 4.5 and 5.9 mmol O_2 l^{-1} h^{-1}. In continuous culture, the preference of substrate was shifted toward laminaran. Industrial production of ethanol from seaweed would require an optimisation of the extraction process, to yield a higher ethanol concentration. The process would be most economic in combination with a total utilization of the seaweed material.

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