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High Productivity Fermentation for Ethanol Production

Kye Joon Lee
Korea Institute of Science and Technology
P.O. Box 131, Dongdae-mun, Seoul, Korea
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高生産性 Ethanol 醱酵에 関하여

李 啓 準
韓國科學技術院
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1. Introduction

The current interest in ethanol as a potential liquid fuel or fuel supplement has stimulated several kinds of research on improving productivity of ethanol fermentation. In order to gain some perspective on fermentations for high productivity, it is important to review the current status of ethanol fermentations using yeasts and to explore the possibility of using another type of microorganisms. The review will assess firstly both the unique properties of yeasts which make them suitable organisms for ethanol production and the various fermentation systems which have been designed to exploit these properties. Following some studies with conventionally used strains of yeast, *Zymomonas modilis*, a promising alternative to yeast as a ethanol producer will be assessed.

2. Ethanol fermentation technology using yeasts

2.1. Properties of yeasts relevent to ethanol fermentation

Yeasts have a number of properties useful for ethanol production. These include:

(1) the ability to ferment and grow in relatively high sugar concentrations (250-400 g/l and higher for osmotolerant yeasts)
(2) the ability to withstand relatively high ethanol concentrations (even up to 200 g/l ethanol in Sake fermentation although this is a slow process occurring at relatively low temperature)
(3) relatively high yields of ethanol from glucose and other sugars (values have been reported as high as 80 to 95% theoretical)
(4) the ability to use a wide range of sugars (e.g. glucose fructose, sucrose, maltose, lactose etc.) and even starch. The metabolism of starch however is relatively slow and is specific to certain strains of yeast (e.g. *S. diastaticus*). This strain-specificity is also true with some of the sugars (e.g. lactose utilization by *Kluyveromyces fragilis*).
(5) the by-product value of yeasts is established. Most yeasts used to produce ethanol have been proven as additive to animal feeds (with no toxicologcal problems) and have crude protein contents of 40-55% dry weight.

The recent renewed interest in fermentation ethanol has stimulated research into isolation and development of improved strains of yeasts. Ethanol and sugar tolerant strains are likely to be useful for industrial ethanol production. For example, Rose[1] selected an ethanol and sugar tolerant strain of *S. uvarum* which was able to ferment completely 250 g/l sugars and produce 106 g/l

Table 1. Kinetic parameters for various strains of yeasts used for ethanol fermentation.

Microganism	Culture system	Clucose input (g/l)	Kinetic parameters at optimal condition					Reference
			μ (h^{-1})	q_p (g/g/h)	q_s (g/g/h)	$Y_{p/s}$ (g/g)	$Y_{x/s}$ (g/g)	
S. cerevisiae ATCC 4126	Continuous Culture	100	0.17	0.58	1.41	0.41	0.12	Cysewski and Wilke[15]
		89	0.19	0.60	1.39	0.43	0.13	
	Continuous Cell Recycle	100	—	0.58	1.32	0.44	—	Cysewski and Wilke[16]
S. cerevisiae NRRL-Y132	Continuous Culture	100	0.12	0.61	1.38	0.44	0.08	Tyai and Ghose[28]
		220	0.12	0.62	1.32	0.47	0.09	
	Continuous Cell Recycle	100	—	0.67	—	—	—	Ghose and Tyagi[27]
S. uvarum ATCC 26602	Batch	260	0.23*	1.15*	3.05*	0.38	—	Lee *et al.*[4]
	Continuous Cell Recycle	200	—	0.75	1.67	0.43	—	del Rosario *et al.*[29]

* Parameters were estimated by computer simulation where no ethanol inhibition occurred

ethanol. This strain was selected also the basis of its flocculent properties. The kinetic parameters of various yeasts which have been studied for ethanol production are given in Table 1.

In an attempt to improve the ethanol yield of *S. uvarm*, respiratory dificient mutant (petites) were produced using various mutagens. Some petites of *S. uvarum* were found to have ethanol yields nearly twice as high as those of the parent strain ($Y_{p/s}$ = 0.17-0.22) due to the lower biomass formation. However significant problems still remain using this approach due to the increased fermentation time and the decreased cell viability (Bacila and Horii).[2]

Other desirable properties of commercial yeasts are their ability to flocculate (useful for cell recycle and recovery) and to ferment at temperature of 40-50°C (Navarro and Durand,[3] Lee *et al.*[4]). This latter characteristic is valuable when vacuum fermentation or simultaneous saccarification/fermentation of starch or cellulose substrates are contemplated.

2.2. Ethanol inhibition in yeasts

There have been many reports of inhibition in yeasts at increased ethanol concentrations including studies on the kinetics of ethanol inhibition (Holzberg *et al.*,[5] Aiba *et al.*,[6] Bazua and Wilke,[7] Ghose and Tyagi,[8] Lee *et al.*,[4] Moulin *et al.*,[9] Navarro,[10] Righelato *et al.*,[11] Brown *et al.*,[12]).

Loss of viability in yeasts at high ethanol levels has been reported also although the effect was partially alleviated by the presence of dissolved oxygen (Nagodawithana *et al.*,[13] Nagodawithana and Steinkraus,[14] Cysewski and Wilke.[15,16]) The loss of viability may have resulted from accumulation of ethanol within the cell and it has been reported that ethanol levels within the cell can reach much higher concentrations than in the external environment (Nagodawithana and Steinkraus,[14] Negodawithana *et al.*,[17] Navarro and Durand,[3] Navarro,[10] Novak *et al.*[18])

Some of the proposed mechanisms for ethanol inhibition are as follows:

(1) inhibition of solute transport system (Thomas and Rose,[19])

(2) alteration in membrane fluidity and permeability (Ingram,[20] Navarro and Durand,[3] Navarro,[10] Novak *et al.*,[18])

(3) feed-back inhibition on the key enzymes of the glycolytic pathway (Nagodawithana *et al.*,[13], Nagodawithana *et al.*,[17])

(4) physical denaturation of certain enzymes and pro-

teins in the cell

Various techniques have been tried to enhance ethanol tolerance. It has been found, for example, that to maintain cell viability under anaerobic conditions yeasts require the addition of unsaturated fatty acids (oleic and/or linoleic acid) and possibly sterols (ergosterol, cholesterol or stigmasterol, etc.) to the growth medium. Under these conditions some reduction in ethanol inhibition occurred possibly due to increased fluidity and permeability resulting from membrane alteration (Hayashida et al.,[21] Hossack et al.,[22] Thomas et al.,[23] Taylor,[24] Watson and Rose,[25] Hayashida and Ohta.[26]) As mentioned earlier, maintaining a significant level of dissolved oxygen could also sustain cell viability by facilitating intracellular metabolism of some of the above compounds.

2.3. Fermentation systems developed with yeasts

2.3.1. Batch and fed-batch culture

Rapid ethanol fermentations have been achieved using batch and fed-batch cultures by using relatively high cell densities. For example, Nagodawithana *et al.*,[13,14] reported that 95 g/l ethanol could be produced from 25° Brix honey solution in 2-3 h using 8 x 10^8 cell/ml of *S. cerevisiae*. Similarly Ghose and Tyagi[27] found that 95 g/l ethanol could be produced from bagasse hydrolysate (200 g/l reducing sugars) in 6 hour by using an initial concentration of 23.6 g/l *S. cerevisiae*.

2.3.2. Continuous culture

Continuous culture studies without cell recycle have been reported by several groups (Cysewski and Wilke,[15] Rosen,[30] ghose and Tyagi,[27] Righelato *et al.*[11]) While the continuous culture system is useful for studies on yeast physiology, the relatively low volumetric productivities of 4-7 g/l are not attractive commercially. Steady state conditions in continuous culture require that cell growth can occur and for this reason the ethanol concentrations reported by most workers were low 40-45 g/l compared to those attained in batch or fed-batch cultures. However, in the recent study by Righelto et al.,[11] it was reported that a strain of *S. uvarum* ATCC 26602 could sustain 94 g/l of ethanol in continuous culture at a dilution rate of 0.01 h^{-1}.

2.3.3. Continuous culture with cell recycle

High concentrations of yeast cells and hence relatively high productivities of ethanol fermentation have been achieved by cell recycling using either centrifugation or sedimentation. Ethanol productivities of 29 to 36 g/l/h have been reported for this system (Cysewski and Wilke,[15] del Rosario *et al.*,[29] Ghose and Tyagi.[27]) With effective recycle systems and strongly flocculent strains, it should be possible to increase yeast concentration to as high as 100 g/l with ethanol productivities up to 50 g/l/h. However significant problems of cell viability and toxic product accumulation occur at such high cell densities.

A relatively simple system for cell recycle is a tower fermenter using high density flocculent yeasts (Hough and Button,[31] Prince[32]). However this system has not been fully evaluated for ethanol production and may be limited by stability considerations.

2.3.4. Vacuum fermentation

If the cell density can be adequately increased by cell recycle, ethanol inhibition becomes the limiting factor as more concentrated sugar solutions are used. Removal of ethanol during the fermentation should give considerable increases in productivity. In this context laboratory scale vacuum fermentation have been carried out by Ramalingham and Finn[33] and by Cysewski and Wilke.[16] The results of the latter authors established that high ethanol productivities (in excess of 80 g/l/h) could be achieved. It has been claimed furthermore that the energy requirements for this system were not appreciably greater than for a conventional process (Maiorella and Wilke[34]) although this assumption has been challenged (Ghose and Tyagi[28]).

2.3.5 Immobilized yeast cell reactors

A multistage system or plug flow fermentor has the advantage of minimizing the effect of ethanol inhibition which occurs only in the later stages in the system (Moreno and Goma[37]). Several groups have reported the use of immobilized yeast cell reactors to increase ethanol productivity. However most of data reported were collected from laboratory scale units and problems may arise during scale-up due to cell growth and gas removal due to significant CO_2 evolution during fermentation.

As far as the technique of immobilization is concerned a number of different supports has been used. These include Ca-alginate (Kierstan and Bucke,[38] White and

Portno,[39] Larson and Mosbach,[40] Cheetam *et al.*[41]) K-carrageenan gel (Chibata and Tosa,[42] Wada *et al.*[43]), gelatine cross-linked with glutaraldehyde (Griffith and compere,[44] Sitton *et al.*,[45]), celite or bentonite (Baker and Kirsop,[46] Grinbergs *et al.*[47]), porous ceramics, bricks, glass or PVC (Marcipar *et al.*[48] Corrieu *et al.*,[49] Navarro and Durand[50]), and inert cellulose-based supports (Ghose and Bandyopadhyay,[51] Moo-Young *et al.*[52]).

Yeast viability has been maintained surprisingly well in such systems. Ghose and Bandyopadhyay,[51] for example, reported that stable operation for over 75 days was possible. Wada *et al.*,[43] reported that no deterioration of activity was observed over 3 months operation. The concentration of ethanol in an effluent was relatively high. Wada *et al.*,[36] for example, reported that production of ethanol of 114 g/l was maintained at a retention time of 2.6 h over 2 months with a conversion rate of glucose to ethanol 20 and 80 g/l/h have been reported and these are comparable and even better than those achieved in cell cycle fermentations. The comparative performance data and ethanol productivities for various fermentation systems using yeasts are given in Table 2.

3. Development of high productivity fermentation systems using *Zymomonas mobilis*

3.1. Properties of *Z. mobilis* as an ethanol producer

From the extensive review by Swings and DeLey,[53] it was evident that strains of *Z. mibilis* has a number of interesting properties for ethanol production. These included:

(1) the ability to ferment sugars rapidly and to tolerate high concentration of sugar

(2) the ability of a number of strains to tolerate relatively high concentrations of ethanol and

Table 2. Comparison of various fermentation systems with yeasts for ethanol production

System used	Strain used	Conc^n of glucose (g/l)	Conc^n of cell (g/l)	Conc^n of ethanol (gl/)	Retention time (h)	Productivity (g/l/h)	Reference
Batch	*S. cerevisiae*	25° Brix	8 x 18^8 (cell/ml)	95	3	31.6	Nagodawithana *et al.*[14]
	"	220	26	95	4.5	21.6	Ghose and tyagi[27]
Ped batch	*S. uvarum*	160-360	83	75	5.0	25.0	del Rosario *et al.*[29]
Continuous	*S. cerevisiae*	89	12.5	41	5.9	7.0	Cysewski and Wilke[16]
	"	160	3.9	33	8.0	4.1	Ghose and Tyagi[27]
Immobilized cell	*S. cerevisiae*	175	—	70	4.5	15.6	Linko and Linko[35]
	"	250	—	114	2.6	32.6	Wada *et al.*[36]
Cell recycle	*S. cerevisiae*	150	50.0	46	1.58	29.0	Cysewski and Wilke[16]
	S. uvarum	200	48.0	60	1.66	36.0	del Rosario *et al.*[29]
Vacuum recycle	*S. cerevisiae*	334	124.0	100-160*	—	82.0	Cysewski and Wilke[16]
no recycle			50.0		—	40.0	

* ethanol concentration in the condensate of vapour stream.

(3) the potential for high yields of ethanol and low yields of biomass.

culture of *Zymomonas* have been isolated historically from pulque and tained cider or beer. The naming this bacterium as *Zymomonas* was adapted in Bergey's manual 7th ed.[54] with two species *Z. mobilis* and *Z. anaerobia.* However Swings and Deley,[53] reclassified this genus as follows: *Z. mobilis* subsps mobilis and *Z. mobiis* subsps *pomaceae.*

The metabolic pathway of sugars in this bacterium follows the Entner Doudoroff Pathway to produce 1.8 mole of ethanol, 1.9 mole of CO_2 and trace of lactic acid from 1 mole of glucose metabolized (Kluyver and Hoppenbrouwers).[55] The hydrolysis of sucrose to glucose and fructose with concomitant formation of levan (polymer of fructose subunit) is the first step in sucrose metabolism (Ribbon *et al.*[56] and Dawes *et al.*[57]).

3.2 Selection of suitable strains for ethanol production

A number of a strains of *Zymomonas* has been evaluated for the selection of suitable strains for high productivity ethanol fermentations and for further genetic studies.[58] A comparison of the rates of growth and ethanol production by eleven different strains of *Zymomonas* revealed a wide range of characteristics as shown in Table 3. Some strains were more tolerant of high sugar or ethanol concentrations and high incubation temperatures than others. Some strains were not able to utilize sucrose; others produced large amounts of levan, and one strain (Ag 11) grew well but produced no levan.

Strain comparison studies were carried out also by Viikari *et al.*[59] who tested *Z. mobilis* ATCC 10988 and seven other *Zymomonas* strains in order to evaluate their ability to produce ethanol in a simultaneous saccharification/fermentation process.

3.3 Comparison of kinetic parameter of *Z. mobilis* with yeasts

A comparison of the kinetic parameters of strains of *Z. mobilis* and strains of *S. cerevisiae* and *S. uvarum* was reported by Rogers *et al.*[60,61] and Lee *et al.*[62-64] The data are shown in Table 4. It is clear that *Z. mobilis* has a number of characteristics which could give it an advantage over yeast for future commercial ethanol production. These include:

(1) higher ethanol and lower biomass yield on glucose and fructose media; this is indicative of a higher efficiency for ethanol production from glucose and frcutose, but not sucrose with the present strains
(2) higher specific rates of glucose uptake and ethanol production at relatively high concentrations of ethanol
(3) higher specific growth rate at relatively high concentrations of glucose and ethanol
(4) ability to grow anaerobically and not to require the controlled addition of oxygen for maintaining cell viability.

3.4 Effect of ethanol inhibition on fermentation kinetics of *Z. mobilis*

As described in previous, ethanol inhibition on the growth of ethanol producing microorganisms and more significantly on the specific rate of ethanol production is a very important factor for high productivity of ethanol production. The effect of ethanol inhibition on the specific growth rate (μ) and specific glucose uptake rate (qs) are shown in Figure 1 for strain of *Z. mobilis*.[63] It was evident that there existed a range of ethanol concentrations (viz 86-127 g/l) in which growth no longer occurred but for which glucose uptake and ethanol production continued. This observation provides further concept that uncoupling of growth and ethanol production in *Z. mobilis* can be initiated by a number of factors including high levels of ethanol concentrations. These characters were considered as an advantage over the yeast for high productivity ethanol fermentation using cell recycling system, since the specific rate of ethanol production in yeasts was inhibited to reduce significantly by increasing of ethanol concentration.

As an ethanol producer strains of *Z. mobilis* is clearly superior and it might be considered that one objective of strain selection programme would be to isolate culture with elevated threshold ethanol concentrations which initiate to inhibite the specific rate of ethanol production. Further effect of ethanol concentration on cell viability is also of paramount importance. Once the threshold concentration of ethanol has been exceeded both inhibition effects (on μ and q_p), then changes in cell viability will influence the fermentation kinetics.[65]

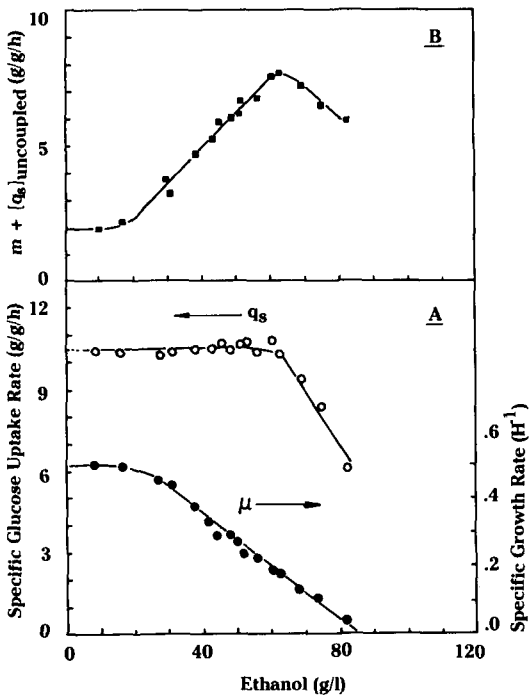

Fig. 1. (A) Effect of ethanol on the specific growth rate and glucose uptake of *Z. mobilis* ZM4.
(B) Effect of ethanol on the combined maintenance energy co-efficient and uncoupled glucose uptake in glucose-excess continuous culture.

3.5 Strain improvement for industrial applications

Since strain ZM4 was clearly best one for ethanol production in all aspects (Table 3), but there was still room for further improvement of, for example, ethanol tolerance, attempts were made to genetically manipulate ZM4 (Skotnicki *et al.*)[66] Strains of *Z mobilis* have a low level of spontaneous mutation rate (viz occurrence of rifampicin resistance was about 10^{-8}) and no increase in this level could be detected after exposure to UV light. However, with nitrosoguanidine (NTG) as the mutagen, a tenfold increase in the rate of mutation was reported (Skotnicki *et al.*[66]).

3.6 Development of fermentation systems for high productivity

In the present research, one major objective has been to evaluate various improved strains of *Z. mobilis* viz. ZN4 and ZM481, and to maximize the productivity of a continuous cell recycle system using these improved strains. In parallel studies within the group, other researchers have evaluated alternative modes of fermentation including cell immobilization[67] semi-batch fermentation using a flocculent strain of *Z. mobilis* to facilitate settling (Lee J.H. unpublished results) and vacuum fermentation (Lee J.H. *et al.*[68]). A comparison of the various systems is given in Table 4, and it is evident that both a high productivity and a relatively high ethanol concentration are desirable for economic operation.

High concentrations of ethanol could be produced in batch culture, which would offer an advantage in lowering product recovery costs. However, it was considered that the ethanol productivities were far too low to be competitive with other systems. As shown in Table 5 the problem of low productivity can be overcome in semi-batch culture using a flocculent mutant of ZM4 (viz. ZM401). Providing the problems associated with scale-up and the use of commercial raw materials can be overcome, semi-batch culture with *Z. mobilis* may well offer the best potential for future commercialization of the process, at least in the initial stages.

In continuous culture the optimal conditions for the growth of *Z. mobilis* could be maintained at close to the maximal specific rate of ethanol production. Higher ethanol productivities were obtained compared to batch cultures, however the steady state ethanol concentrations were significantly lower.

High ethanol productivities were sustained in a cell recycle system (viz. 120-200 g/l/h) and strain improvement gave rise to relatively high ethanol concentrations (viz. 85-90 g/l).[69] At optimal conditions, the specific rates of ethanol production were maintained at their maximal rates. At 85-90 g/l ethanol, high cell viability was also maintained. It would seem that the effective development of such a system commercially with depend on suitable stable and low cost techniques being introduced for cell recycle.

Immobilized cell reactors have been considered as potential systems for commercial ethanol production due to their simple operation, low cost installation and their facility for minimizing ethanol inhibition effects due to their plug flow character. However the ethanol produc-

Table 3. Comparative data for evaluation of different strains of *Z. mobilis* and *Z. mobilis* subsp. *pomaceae* (Skotnicki *et al.*,[68])*.

Strain used	Source of strain	Specific growth rate at 100 g/l sugar (h^{-1})		Ethanol productivity on 100 g/l (g/l/h)		Max. ethanol concentration from 200 g/l glucose (g/l)		
		glucose	sucrose	glucose	sucrose	30°C	37°C	42°C
Z. mobilis								
ZM1 (ATCC 10988)	V.B.D Skerman	0.22	0.20	1.28	1.16	60	40	0
ZM4 (CP4)	Swings & DeLey	0.25	0.23	1.68	1.25	81	52	30
ZM6 (ATCC 29191)	Swings & DeLey	0.29	0.22	1.04	1.46	77	0	0
Ag 11	Swings & DeLey	0.24	0.19	1.30	1.20	55	0	0
3 TH Delft	Swings & DeLey	0.20	0.19	1.10	1.18	—	—	—
B 70	Swings & DeLey	0.18	0.18	1.10	1.25	—	—	—
ZAbi	J. G. Carr	0.17	0.18	0.70	1.25	—	—	—
Z. mobilis subsp. *pomaceae*								
ATCC 29192	ATCC	0.22	0	1.55	0	—	—	—
238	J. G. Carr	0.16	0	0.64	0	—	—	—
S 30.2	J. G. Carr	0.17	0	1.51	0	—	—	—
S 30.A	J. G. Carr	0.16	0	1.33	0	—	—	—

— Indicates not tested. * Classification of genus *Zymomonas* was followed to the suggestion of Swings & DeLey (1977)

Table 4. Comparisons of the kinetic parameters of *Z. mobilis* and Yeast for ethanol fermentation.

Micro-organism	Culture system	Glucose input (g/l)	Kinetic parameters at optimal condition					Reference
			μ (h^{-1})	q_p (g/g/h)	q_s (g/g/h)	$Y_{x/s}$ (g/g)	$Y_{p/s}$ (g/g)	
S. cerevisiae ATCC 4126	Continuous culture	100 89	0.17 0.19	0.58 0.60	1.41 1.39	0.12 0.13	0.41 0.43	Cysewski and Wilke[15]
S. crevisiae NRRL Y-132	Continuous culture	100 220	0.12 0.12	0.61 0.62	1.38 1.32	0.08 0.09	0.44 —	Ghose and Tyagi[27]
S. uvarum ATCC 26602	Batch	250	0.23	1.15	3.02	—	0.38	Lee J. H. *et al.*[4]
Z. mobilis ATCC 10988 (ZM1)	Batch	100 250	0.21 0.13	2.50 2.53	5.47 5.45	0.038 0.019	0.49 0.47	Rogers *et al.*[60]
	Continuous culture	100 150	0.20 0.235	3.2 3.8	6.4 7.6	0.029 0.027	0.50 0.50	Lee *et al.*[62]
Z. mobilis (ZM4)	Batch	100 250	0.35 0.18	5.2 5.4	10.9 11.3	0.032 0.015	0.48 0.48	Lee *et al.*[63]
	Continuous	100 135 170	0.38 0.28 0.24	5.4 5.4 5.1	10.8 10.8 10.5	0.037 0.025 0.022	0.50 0.50 0.48	Lee *et al.*[63]

Table 5. Comparison of ethanol productivities achieved in various culture system with *Z. mobilis* and strains of yeast on glucose medium.

System used	Micro-organism	Glucose input (g/l)	Conc^n of ethanol (g/l)	Ethanol productivity (g/l/h)	Reference
Batch	*Z. mobilis*	250	119	5.9	Roger *et al.*[60]
	S. uvarum	250	109	2.7	del Rosario *et al.*[29]
Continuous	*Z. mobilis*	170	60.0	12.5	Lee *et al.*[63]
	S. cerevisiae	100	41.0	7.0	Cysewski & Wilke[16]
Recycle	*Z. mobilis*	140	70.0	120-200	Lee *et al.*[60]
	Z. mobilis	200	98.0	93.0	Lee *et al.*[69]
	S. cerevisiae	150	60.5	32.0	Ghose & Tyagi[27]
	S. uvarum	200	60.0	36.0	del Rosario *et al.*[29]
Vacuum with cell recycle	*Z. mobilis*	200	180.0*	85	Lee J.H. *et al.*[67]
	S. cerevisiae	334	160.0*	82	Cysewski & Wilke[16]

* Ethanol concentration in vapour stream from vacuum fermentor.

tivities were found to be much less than for the cell recycle system due to mass transfer and diffusion limitations.

The technology of vacuum fermentation has been evaluated also with *Z. mobilis* (Lee J.H. *et al.*[68]) and from the data summarized in Table 5, it is clear that some increase in productivity can be achieved by comparison with a cell recycle culture. Furthermore the vacuum system allows relatively high sugar solutions (350 g/l and higher) to be fermented. However the additional capital and operating costs for a relatively small increase in productivity (approx. 20%) detract from the commercial potential of the system.

In summary, then, it is clear that by using *Z. mobilis* very high productivities can be achieved by comparison with yeast fermentations. From the viewpoint of the commercial potential of the various systems, it would appear that the semi-batch process in association with a flocculent strain offers the greatest immediate promise. In longer term the continuous cell recycle system has advantages despite the obvious scale-up difficulties. Essentially such a process would operate with a highly productive strain of *Z. mobilis* which could maintain cell viability at relatively high ethanol concentrations (e.g. strain ZM481). By operating at high ethanol concentrations (e.g. 85-90 g/l) there would be little if any biomass production as growth would be fully inhibited and ethanol would be produced entirely as a product of the uncoupled metabolism and maintenance energy requirement. This minimization of growth would (no need to remove excess biomass) and would give some additional economic advantage in terms of an increased ethanol yield.[70-72]

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