Chapter 12

Genetically Engineered *Saccharomyces* Yeasts for Conversion of Cellulosic Biomass to Environmentally Friendly Transportation Fuel Ethanol

Nancy W. Y. Ho, Zhendao Chen, Adam P. Brainard, and Miroslav Sedlak

Laboratory of Renewable Resources Engineering (LORRE), Purdue University, 1295 Potter Center, West Lafayette, IN 47907–1295

Ethanol is an effective, environmentally friendly, nonfossil, transportation biofuel that produces far less pollution than gasoline and contributes essentially no net carbon dioxide to the atmosphere. Furthermore, unlike crude oil for the production of gasoline, ethanol can be produced from plentiful, domestic, renewable, cellulosic biomass feedstocks. However, a major obstacle in this process is that cellulosic biomass contains two major sugars, glucose and xylose. *Saccharomyces* yeasts, traditionally used for large scale industrial production of ethanol from glucose, is unable to ferment xylose to ethanol. This makes the use of the safest, most effective microorganism for conversion of cellulosic biomass to ethanol economically unfeasible. In the fall of 1993, we achieved a historic breakthrough in the successful development of genetically engineered recombinant *Saccharomyces* yeast that can effectively ferment both glucose and xylose to ethanol. This paper provides an up-to-date overview of the design, development, and continuous innovative perfection of our recombinant *Saccharomyces* yeast that is widely regarded as the microorganism which will make the conversion of cellulosic biomass to ethanol commercially possible.

Introduction

Numerous studies have proven that ethanol as a transportation fuel produces less air pollutants than gasoline. This environmentally friendly liquid fuel can be used

© 2000 American Chemical Society
directly as a neat fuel (100\%) or as a blend with gasoline at various concentrations. The raw material used for the production of ethanol fuel is renewable and abundantly available domestically. Thus, the use of ethanol to supplement or replace gasoline not only reduces air pollution and ensures a cleaner environment, but also reduces the dependency of our nation on imported foreign oil, protects our nation’s energy security, and reduces our nation’s trade deficit due to imported oil for the production of gasoline.

Ethanol has been produced by fermenting glucose-based food crops, such as cane sugar, corn starch, and other starch-rich grains, using yeasts, particularly *Saccharomyces* yeasts, which remain the only microorganisms used for large scale industrial ethanol production since the pre-industrial age. However, these agricultural crops are expensive and in limited supply.

Cellulosic biomass, which includes agriculture residues, waste streams from agricultural processing, sugarcane bagasse, municipal solid wastes, yard and wood wastes, wastes from paper mills, etc. is an attractive feedstock for ethanol-fuel production by fermentation because cellulosic biomass is not only renewable and available domestically but also available at low cost and in great abundance. However, there are problems which must be solved for such ideal feedstocks to be economically converted to ethanol. One serious problem is that the major sugars derived from cellulosic biomass include not only glucose but also xylose with a ratio of glucose to xylose approximately 2 or 3 to 1. It is generally agreed that unless both glucose and xylose from the cellulosic biomass could be fermented, the economics of converting cellulosic biomass to ethanol are not favorable. Yeasts, particularly *Saccharomyces*, which have traditionally been used and are still the only microorganisms used by industry for fermenting glucose-based feedstocks to ethanol, are unable to ferment xylose or utilize xylose for growth. It has been found that these yeasts are missing the enzyme(s) that are responsible for the conversion of xylose to xylulose (Figure 1). Furthermore, there are no other naturally occurring microorganisms known that are capable of effectively converting both glucose and xylose to ethanol. Thus, the major obstacle that prevents the economical conversion of cellulosic biomass to ethanol has been the lack of microorganisms, particularly yeasts, that can effectively convert both glucose and xylose to ethanol.

Two decades ago, a great deal of efforts worldwide were devoted to finding new yeasts, particularly *Saccharomyces* yeasts, capable of effectively fermenting both glucose and xylose. However, none were found. Nevertheless, a few other yeasts such as *Pichia stipitis*, *candida shehatae*, and *Pachysolen tannophilus* were discovered which could ferment xylose and utilize it for growth. Unfortunately, these yeasts are not effective fermentative-microorganisms and are not suitable to industry for large scale ethanol production because they are not user-friendly.

More than a decade ago, after exhausting all other means, the scientific community worldwide turned to genetic engineering to develop effective *Saccharomyces* yeasts for fermenting xylose to ethanol. Nearly ten research groups (at least five of them in the United States, including our group) initiated a worldwide attempt to genetically engineer *Saccharomyces* yeasts for xylose fermentation. Without exception, each group initially cloned a xylose isomerase gene from different bacteria into the *Saccharomyces* yeasts to make the latter ferment xylose (Figure 1). However, all efforts to express a bacterial xylose isomerase gene in the *Saccharomyces* yeasts failed to produce a genetically engineered *Saccharomyces* yeast that could
The xylose metabolic pathways in microorganisms.

- Xylose non-utilizing yeasts (*Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, etc.)
- Xylose utilizing yeasts (*Candida shehatae*, *Pichia stipitis*, *Pachysolen tannophilus*, etc.)
- Bacteria (*E. coli*, *Bacillus species*, *Streptomyces species*, etc.)

*Figure 1. Xylose metabolic pathways in different types of microorganisms.*
ferment xylene. After these unsuccessful attempts to clone a xylene isomerase gene in yeast, all U. S. groups except our group at Purdue University gave up on this difficult undertaking. Most experts by then had proclaimed that perhaps it was not possible to genetically engineer any *Saccharomyces* yeasts to effectively ferment xylene to ethanol. They reasoned that the only remaining way to genetically engineer the *Saccharomyces* yeast to ferment xylene was to transfer the genes for the conversion of xylene to xylulose; namely the xylene reductase gene (*XR*), encoding xylene reductase (*XRtase*), and the xylitol dehydrogenase gene (*XD*), encoding xylitol dehydrogenase (*XDNase*), from *Pichia stipitis*, the best natural xylene-fermenting, non-*Saccharomyces* yeast (Figure 1). As shown in Figure 1, the Pichia as well as all the natural xylene-fermenting non-*Saccharomyces* yeast do not have an adequate system to provide cofactors to the *XRtase* and *XDNase* (*1*). On one hand, *XRtase* can use both NADPH and NADH as its cofactor but has much stronger affinity towards NADPH than NADH. On the other hand, *XDNase* requires NAD for its sole cofactor. In yeast there is no proper enzyme system to convert NAD to NADPH or vice versa, particularly under anaerobic conditions. Thus, most scientists predicted that xylene fermentation with such a genetically engineered yeast would stop due to lack of the proper cofactors. Furthermore, it was known that the *XDNase* catalyzes the interconversion between xylulose and xylitol but favors the formation of xylitol (the reverse reaction), which is compounded by the fact that most *Saccharomyces* yeasts have very low levels of xylulokinase activity (*2*). Thus, most experts concluded that even if one succeeded in cloning the *XR* and *XD* genes from *P. stipitis* into a *Saccharomyces* yeast, the engineered yeast would produce xylitol rather than ethanol.

Instead of abandoning this important project we carefully planned our approach to not only overcome all the above problems but also to design our engineered yeasts with additional properties that would make them ideal for fermenting glucose and xylene present in cellulosic biomass to ethanol. This paper presents an up-to-date overview of the design, development, and continuous innovative perfection of our genetically engineered recombinant *Saccharomyces* yeasts that can not only effectively ferment xylose to ethanol but also simultaneously coferment glucose and xylene to ethanol.

**Design and Development of an Ideal Yeast for Effective Cofermentation of Glucose and Xylose to Ethanol**

The uniqueness of our approach is that the yeasts engineered to not only ferment xylose but also (i) to effectively direct the metabolic flux towards the production of ethanol rather than the production of byproducts such as xylitol, (ii) to overcome the natural barrier "glucose effect," making it possible for the resulting engineered yeast to effectively coferment both glucose and xylene simultaneously so that the mixed sugars will be fermented as fast as possible, (iii) to easily convert most *Saccharomyces* strains, particularly the superior glucose-fermenting industrial strains to coferment xylose in addition to glucose, (iv) to use rich medium for growth and fermentation to make the engineered yeast grow and ferment sugars faster, and (v) to solve the potential waste problems by recycling used yeast cells for the production of crude yeast extracts for culturing new yeast cells. Furthermore, the final genetically engineered yeasts are stable and can be used in either batch or continuous process for
ethanol production without the use of special chemicals as selection pressure to maintain the cloned genes.

In order to accomplish all of the above, we cloned not only a xylose reductase gene \((XR)\) and a xylitol dehydrogenase gene \((XD)\) but also a third gene, the xylulokinase gene \((XK)\), even though all the \textit{Saccharomyces} yeasts already contain a functional \(XK\). We also replaced the signal sequences that control the expression of the three cloned genes with sequences that control the expression of yeast glycolytic genes.

After over ten years of dedicated research, our group at Purdue University achieved a historic breakthrough in the fall of 1993. As the first and still the only research group in the world to overcome the aforementioned obstacles, we succeed in the development of genetically engineered \textit{Saccharomyces} yeasts that effectively ferment both glucose and xylose present in cellulosic biomass to ethanol. This was accomplished by first developing several stable plasmids such as pLHN32 and pLHN33 (Figure 2) that contain the three cloned xylose-metabolizing genes \(XR\), \(XD\), and \(XK\), all fused to glycolytic promoters and subsequently designated as \(AR\) (or \(A*R\)), KD, and KK (for details see ref. 3). These recombinant plasmids were constructed by closely following the design outlined above. They performed as expected. For example, these plasmids are able to transform various xylose non-utilization yeasts, particularly the \textit{Saccharomyces} yeasts, and convert them to genetically engineered yeasts that are able to effectively coferment both glucose and xylose to ethanol. Yeast strain 1400 is one of the \textit{Saccharomyces} yeasts that have been transformed with pLHN32 and pLHN33. The resulting genetically engineered yeasts, designated 1400(pLHN32) and 1400(pLHN33) (also referred to as LHN32 and LHN33), can effectively coferment glucose and xylose to ethanol. For example, 1400 (pLHN32) can ferment 8% glucose and 4% xylose mostly to ethanol in 48 hr as shown in Figure 3. The parent yeast, strain 1400, can ferment only glucose but not xylose to ethanol (Figure 4) (3).

Our genetically engineered \textit{Saccharomyces} yeasts have outstanding properties that make them ideal for the industrial production of ethanol. For instance, they can effectively ferment glucose and xylose both separately and simultaneously to ethanol as shown in Figure 3. This is an extremely important property for economical production of ethanol from biomass. Furthermore, our engineered yeasts are very stable and can use rich medium for fermentation and growth. They do not require the presence of expensive or undesirable chemicals, such as antibiotics, to maintain the cloned genes for fermentation of xylose. In addition, our genetically engineered yeasts produce very little byproduct such as xylitol (Figure 3) and can grow very well in medium using either glucose or xylose as the sole carbon source, as shown in Table 1.

The Effect of Cloning and Overexpression of the Xylulokinase Gene

One of the major differences between our genetically engineered \textit{Saccharomyces} yeasts and those reported by others (Kotter et al., Walfridsson et al., and Tantiumpikul et al.) (4-6) is that in addition to cloning the \(XR\) and \(XD\) genes, we also cloned and overexpressed the \(XK\) genes, even though nearly all \textit{Saccharomyces} yeasts have an active xylulokinase gene and produce an active xylulokinase enzyme in the presence of
Figure 2. Restriction map of the PLNH plasmids. (Reproduced from reference 3. Copyright 1998 American Society for Microbiology).
Figure 3. Cofermentation of glucose and xylose by genetically engineered yeast 1400(pLNH 32). (Reproduced from reference 3. Copyright 1998 American Society for Microbiology.)
Figure 4. Cofermentation of glucose and xylose by the parent yeast strain 1400. (Reproduced from reference 3. Copyright 1998 American Society for Microbiology.)
3. Same as 2 except 2% xylose instead of glucose.
2. 1% yeast extract, 2% peptone, 2% glucose.
1. KU = Klier Unilabs, the optical density units measured by Klier-Sunismeron photodensitometer.

<table>
<thead>
<tr>
<th></th>
<th>17</th>
<th>11</th>
<th>39</th>
<th>19</th>
<th>18</th>
<th>17</th>
<th>17</th>
<th>17</th>
<th>17</th>
<th>17</th>
<th>17</th>
<th>17</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parent Yeast</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>520</td>
<td>482</td>
<td>116</td>
<td>99</td>
<td>30</td>
<td>18</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>425</td>
<td>400</td>
<td>205</td>
<td>87</td>
<td>35</td>
<td>19</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>550</td>
<td>400</td>
<td>122</td>
<td>68</td>
<td>37</td>
<td>21</td>
<td>18</td>
<td>18</td>
<td>18</td>
<td>18</td>
<td>18</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>472</td>
<td>455</td>
<td>289</td>
<td>120</td>
<td>44</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>21</td>
</tr>
</tbody>
</table>

<p>| | | | | | | | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>KU</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Comparison of the Growth of Recombinant Saccharomyces and the Parent Yeast in Glucose and Xylose
xylose. As a result, our genetically engineered yeast can effectively utilize xylose for growth and ferment xylose to ethanol. On the contrary, those developed by Kotter et al. (4) and Walfridsson et al. (5) are neither able to utilize xylose for growth nor able to ferment xylose to ethanol. Although Tantirungkij et al. (6) reported that their engineered yeast can grow with xylose as the sole carbon source, their yeast still cannot ferment xylose to ethanol. It appears that overexpression of the xylulokinase gene makes it possible to overcome the problems of cofactor imbalance and the favoring of xylitol production as described above.

To demonstrate the effect of cloning $XK$ on metabolizing xylose by the *Saccharomyces* yeast, we constructed a new plasmid pUCKm10-XR-XD which is identical to pLNH33 except that it lacked the cloned $XK$ gene. *Saccharomyces* strain 1400 transformants containing pUCKm10-XR-XD utilized xylose for growth at much slower rates, less than one-fifteenth the rate of the same yeast strain containing pLNH33. Furthermore, the genetically engineered 1400 yeast containing pUCKm10-XR-XD, designated 1400(pXR-XD), could only convert xylose to xylitol, but not ethanol as illustrated in Figures 5A and 5B. Preliminary results of this work have been published elsewhere (7).

**The Effect of Replacing the Original Promoters of the cloned $XR$, $XD$, and $XK$ Genes with *Saccharomyces* Glycolytic Promoters**

Nearly all naturally occurring microorganisms, including the *Saccharomyces* yeasts, only metabolize glucose when a mixture of sugars, such as glucose and xylose, are present in their media. Furthermore, microorganisms require the induction of new enzymes to use another sugar, for example xylose, after glucose is depleted from their media. Thus, there usually is a lag period after glucose has been depleted before the second sugar can be actively used by a microorganism, a phenomenon generally known as the "glucose effect". In our view, the time required for microorganisms, including yeasts, to convert a feedstock containing mixed sugars to ethanol would be greatly shortened if the microorganisms were made free from the glucose effect and also the requirement that xylose be present for the induction of the xylose-specific enzymes. We determined that this could be accomplished by replacing the regulatory sequences controlling gene expression (present within the 5' noncoding sequences) of those genes specifically required for metabolism of xylose with regulatory sequences not subject to the glucose effect. Besides the $XR$ and $XD$ genes, the $XK$ gene is also subject to the glucose effect and requires the presence of xylose for induction of the synthesis of its protein. Thus, this is another reason why it is necessary to clone the xylulokinase gene, even if the parent yeasts contain high levels of xylulokinase activity.

In order to prove that cloning KD, AR or A+R, and KK (XD, XR, and XK structural genes fused to glycolytic promoters) is extremely important for cofermentation of feedstocks (or media) containing mixtures of glucose and xylose, we compared the fermentation of a mixture of glucose and xylose by 1400(pLNH 32) and *Pichia stipitis*. As described above, the *Pichia* yeast is a naturally occurring xylose-fermenting yeast that can ferment xylose with similar efficiency at the laboratory scale under well controlled conditions as the genetically engineered 1400(pLNH32). However, the expression of *Pichia's* $XD$, $XR$, and $XK$ genes is subject to glucose
Figure 5. Comparison of fermentation of xylose under identical conditions as described in the Methods of reference 3 by (A) genetically engineered Saccharomyces yeast strain 1400(pLNH32) which contains the cloned and genetically modified XR, XD, and XK genes and by (B) 1400(pXR-XD) which contains only the same cloned XR and XD genes, but not the cloned XK gene.
inhibition and requires the presence of xylose for induction. As shown in Figures 6A and 6B, when these yeasts were cultured in YEPX and used to ferment a mixture of glucose and xylose, 1400(pLNH32) effectively cofermented both glucose and xylose to ethanol, whereas P. stipitis fermented glucose but not xylose to ethanol. Furthermore, our engineered yeast cultured in 50 ml YEPD medium with 2 ml of YEPX pregrown cells as the seed culture is also able to ferment a mixture of glucose and xylose to ethanol with similar efficiency as shown in Figure 6A. Thus, our results demonstrate that in order for microorganism (yeast) to be effective in cofermenting xylose and glucose to ethanol, it must be able to synthesize xylose-metabolizing enzymes in the presence of glucose. Preliminary results of this work have been published (7).

Effective New Gene-Integration Technique for the Development of Superstable Genetically Engineered Saccharomyces Yeasts

The 1400(pLNH32) and related plasmid-mediated genetically engineered xylose-fermenting Saccharomyces yeasts are very stable and their xylose-fermenting ability can be maintained by an ideal selection mechanism (3). However, a more perfect yeast for fuel ethanol production should be completely stable without the need for selection pressure to maintain its xylose-fermenting ability at any stage of growth or fermentation. In 1995, we successfully developed the first superstable strain of genetically engineered glucose-xylose-cofermenting Saccharomyces yeast, 1400(LNH-ST), which requires no selection pressure to maintain its ability to ferment xylose to ethanol (data not shown) or to utilize the sugar for growth as shown in Table 1. Furthermore, this superstable xylose-fermenting genetically engineered yeast coferments glucose and xylose to ethanol with equal or greater effectiveness than the plasmid-mediated recombinant yeasts such as 1400(pLNH32). This superstable genetically engineered Saccharomyces yeast contains multiple copies of the same three modified XYL genes, AR, KD, and KK, integrated into the host chromosome. It is generated by means of a much improved technique for integrating multiple copies of genes into the yeast chromosome, a method that is actually much easier to accomplish than all methods previously described in the literature. At this time, we have completed the development of three stable glucose-xylose-cofermenting Saccharomyces yeast strains, 1400(LNH-ST), 259A(LNH-ST), and 424A(LNH-ST). They are derived from three different parent strains of Saccharomyces yeasts, and they are all efficient in co-fermenting glucose and xylose to ethanol. The use of this unique gene-integration technique allows one to easily control the copy-number of the integrated genes which thereby facilitates development of the ideal Saccharomyces yeasts for ethanol fuel production from cellulosic biomass as envisioned years ago (see Figure 7). According to our original design, Saccharomyces yeasts that are superior for ethanol production from traditional glucose-based feedstocks, or that have strong tolerance to ethanol, high temperature, or inhibitors present in hydrolysates of the cellulosic biomass, should all be successfully genetically engineered by our technology to effectively ferment xylose to ethanol. This unique gene-integration technique makes it possible to convert most, if not all, Saccharomyces yeasts to effectively coferment glucose and xylose to ethanol. It should also make it possible to further improve our engineered yeasts to be able to coferment other sugars present
Figure 6. Comparison of cofermentation of glucose and xylose under identical conditions as described in the Methods of reference 3 by (A) genetically engineered Saccharomyces yeast strain 1400(pLNH32) and by (B) Pichia stipitis. Symbols: square, glucose; circle, xylose; triangle, ethanol.
Figure 7. Stepwise integration of multiple copies of the cloned multiple genes into the host chromosome(s). 259A(LNH-ST)(Int 0) (shown in A) contains fewer copies of the integrated xylose-metabolic genes than 259A(LNH-ST)(Int 3) (shown in B).
in the cellulosic biomass hydrolysates, as well as to produce some high-valued byproducts or co-products in addition to ethanol. The development of these stable genetically engineered glucose-xylose-cofermenting *Saccharomyces* yeasts and the method of integrating multiple copies of gene(s) into the yeast chromosome have been briefly described previously (8) and the details of the development of these stable yeasts will be reported elsewhere.

**Repeated Cofermentation of a mixture of Glucose and Xylose Efficiently to Ethanol**

Recently we determined that our engineered glucose-xylose-cofermenting *Saccharomyces* yeasts can repeatedly coferment a mixture of glucose and xylose efficiently to ethanol for numerous cycles as shown in Figure 8. Moreover, our "stable" glucose-xylose cofermenting yeasts can repeatedly coferment sugars present in hydrolysates of cellulosic biomass including glucose, xylose, mannose, and galactose. Our initial results showed that the engineered yeasts can produce 20%-40% more ethanol than the unengineered parent yeast, depending on the initial pH at each cycle of fermentation (data not shown). The latter results may lead to the development of much more cost-effective processes for the production of cheap ethanol from cellulosic biomass.

**Genetically Engineered *Saccharomyces* Yeasts Cofermenting Glucose and Xylose Present in Crude Hydrolysates**

Our stable strain 1400(LNH-ST) was confirmed to be able to effectively coferment glucose and xylose to ethanol by a continuous process in a 9000-L pilot scale bioreactor for 90 days with both pure sugars and sugars from crude corn biomass hydrolysates as the feedstocks (9). Our genetically engineered *Saccharomyces* yeasts were shown to be able to coferment glucose and xylose present in various other crude cellulosic biomass hydrolysates very effectively (7, 10). Furthermore, our glucose-xylose-cofermenting *Saccharomyces* yeasts can also repeatedly coferment glucose and xylose present in crude hydrolysates of soft wood to ethanol (Ho et al. unpublished results) and undoubtedly these yeasts should also be able to repeatedly coferment glucose and xylose present in crude hydrolysates of other cellulosic biomass.

**Conclusions and Future Perspective**

In this paper, we reviewed our strategies used to genetically engineer the *Saccharomyces* yeasts, resulting in the development of innovative technology that can successfully transform most, if not all, the *Saccharomyces* yeasts from non-xylose-fermenting to xylose-fermenting as well as to glucose and xylose cofermenting species not subject to control by "glucose" inhibition.

Our unique gene-integration technique that can effectively integrate multiple copies of multiple genes into the yeast chromosome has made it possible for us to develop (the perfect) "stable" genetically engineered yeasts that can effectively
Figure 8. Repeated fermentation of glucose and xylose to ethanol by the same batch of genetically engineered microbes-glucose-xylose-fermenting Saccharomyces.

1. Ethanol
2. Xylose
3. Glucose
coferment glucose and xylose to ethanol for hundreds of generations without requiring
the presence of any selection pressure to maintain the three cloned xylose metabolism
genes.

With our reliable and effective gene-integration technique coupled with the most
advanced recombinant DNA techniques available to yeast manipulation, and the most
complete genetic information and data available for the *Saccharomyces* yeasts, our
engineered yeasts can further be improved for the coproduction of numerous other
high-value products. Because *Saccharomyces* yeasts are the safest, hardiest, and most
user-friendly of the industrial microorganisms, there is no doubt that our genetically
gineered *Saccharomyces* yeasts should be and will be the microorganisms of choice
for the cost-effective production of cheap ethanol from cellulosic biomass, just as
traditional *Saccharomyces* yeasts are the microorganisms of choice for the large-scale
industrial production of ethanol from glucose-based feedstocks.

**Acknowledgments**

The authors wish to acknowledge that their current work is supported in part by
the U.S. Environmental Protection Agency and the National Renewable Energy
Laboratory of the U. S. Department of Energy and that their past work was supported
by the U.S. department of Agriculture, the U. S. Department of Energy, and Swan
Biomass Company.

**Literature Cited**

1. Bruinenberg, P. M.; deBot, P. H. M.; van Dijken, J. P.; Scheffers, W. A. Appl.
199.
1852-1859.
493-500.
7. Ho, N. W. Y.; Chen, Z.; Brainard, A. P.; Sedlak, M. in Recent Progress in
Bioconversion of Lignocellulosics; Tsao, G. T. Ed.; Springer-Verlag: Heidelberg,
Germany; in press.
255.
13, 341-346.