Emerging Technologies and Future Prospects for Industrialization of Microbially Derived Cellulose

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This presentation will describe a new application of biotechnology to the production of one of the most widely used biopolymers, cellulose. The new application exploits microbially derived cellulose on an industrial scale. The gram negative bacterium, *Acetobacter xylinum* is the microorganism of choice since it synthesizes copious amounts of cellulose of unsurpassed purity, mechanical strength and absorbability. The kinetics and potential efficiency of microbial cellulose production as well as goals, obstacles, and future strategies will be presented. Optimal fermentation pathways in relation to the final cellulose product characteristic will be described. The importance of molecular biology and genetic engineering for intermediate and long term strategies of the cellulose industry will be discussed.

Cellulose is the most abundant macromolecule on the planet (Brown, 1985). Over 10^{11} tons is made and destroyed annually. Thus, it is of vital interest to applied science to understand how this vast polymeric material is synthesized, for if we can control the biosynthesis of cellulose, we are in a unique position to beneficially alter and promote our global climate as well as produce a major industrial raw material. Considering that cellulose is a sink for atmospheric CO_2, the more gaseous CO_2 we can "fix" into solidified mass, the better we can control the global warming trend.

How should we tackle this problem? The first idea might be to simply stop using our forests and let them grow! We all know that this is not feasible in view of the continuing demand for cellulose from forests and from cotton. Thus, we must turn to other sources of cellulose, and this is where *microbially derived cellulose* can provide a new purpose and direction. Cellulose produced under a directed and controlled *fermentation process* is a biotechnological ideal. Since the gram negative bacterium, *Acetobacter xylinum* is the most prolific synthesizer of microbial cellulose (Brown, 1989), it is the focus of current studies.

What is microbially derived cellulose and what are its advantageous characteristics?

Microbially derived cellulose is *pure* cellulose, without any lignin or other cell wall contaminating material. *Acetobacter xylinum* synthesizes cellulose in the form of a composite ribbon of microfibrils which exit the cell envelope from a distinct row of pores or "spinnerets" (Fig. 1). Since the microfibrils are spun into the growth medium, all cells in the medium contribute to the gross morphology of the cellulose which is in the form of a gelatinous membrane or *pellicle*. This membrane contains entrapped cells and is formed at the gas/liquid interface of the culture medium in static cultures. Typical nutrients are glucose, sucrose, proteose peptone, and yeast extract. Culture medium is inoculated with cells and a visible pellicle usually forms within 24 hrs. Static cultivation for one week or more results in a thick, strong, hydrophilic pellicle.

Cellulose production is active only in a narrow zone of cells at the upper surface of the pellicle. Cells become entrapped within the pellicle as new cells produce overlaying cellulose. Those entrapped cells will eventually die due to oxygen deprivation from above and nutrient deprivation from below. In spite of this limitation, cellulose yields of 45% are not uncommon.

Two properties of the microbial cellulose membrane far exceed those typical of celluloses of vascular plants: (a) the cellulose is very hydrophilic with a water absorption capacity of over 100 times the weight of cellulose; (b) the cellulose has great mechanical strength, far surpassing that of pulp paper and cotton textiles. Given these important characteristics, it is reasonable to expect that microbial cellulose can find a useful place in the industrial market. This is one of the major goals of our microbial cellulose research and development program at The University of Texas at Austin.

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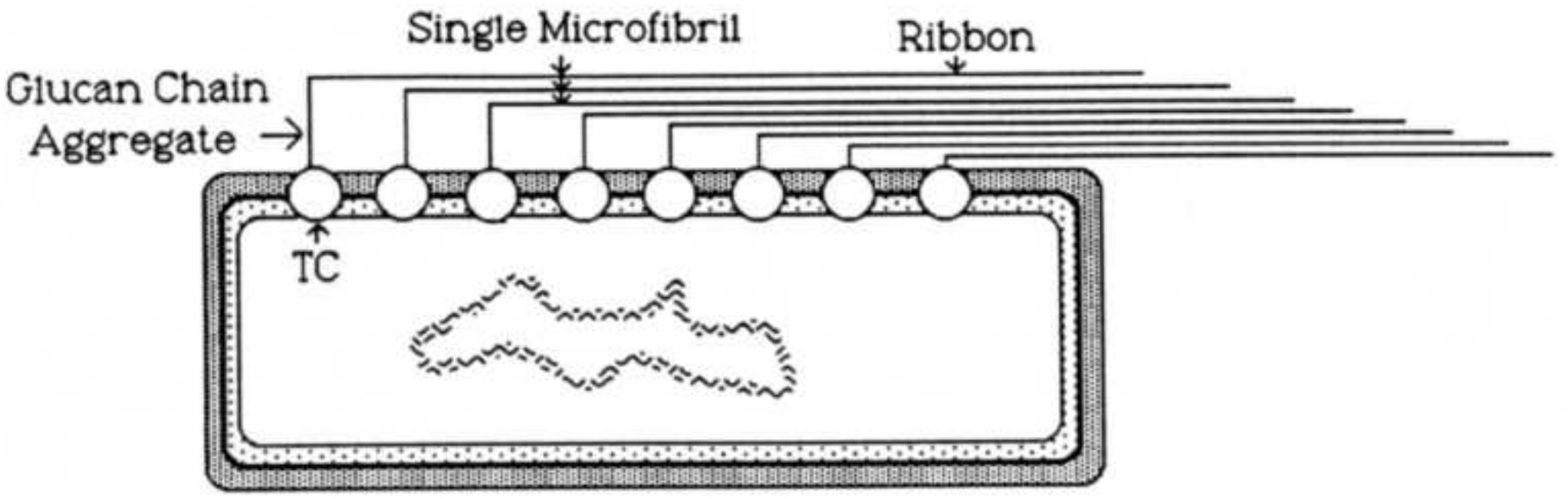

Figure 1. Idealized schematic diagram of an *Acetobacter* cell showing the linear arrangement of the cellulose synthesizing complexes (=TC). Each TC synthesizes a glucan chain aggregates consisting of approximate 6 -8 glucan chains. Three TCs are required to synthesize their respective glucan chain aggregates which are required to form a crystalline microfibril. Microfibrils aggregate via H-bonding to form the cellulose ribbon which is secreted into the medium. The matrix of interwoven ribbons constitutes the bacterial cellulose membrane or pellicle.

Kinetics and efficiency of microbially derived cellulose production

In contrast to cellulose production from a crop such as cotton, microbially derived cellulose has a significantly larger yield. Consider the average yield of one bale of cotton per acre per year as a standard of comparison. Since microbially derived cellulose can be synthesized by living bacterial cells in shallow pans requiring only adequate nutrition and oxygenation, the yield of cellulose from *a single tray* only several mm deep with the corresponding surface area of one acre, has the potential to produce more than 26,000 lb of cellulose per year culture time! This comes to more than 10^8 glucose molecules incorporated into cellulose each hour per cell.

Microbially derived cellulose has yet another unique feature in that it can be substantially modified *during synthesis*. This means that the physical properties of the cellulose can be predetermined by the circumstances of the fermentation, a tremendous advantage over the absence of control of the characteristics of the cellulose produced within a cotton boll or forest trees.

Goals, obstacles, and future strategies for microbially derived cellulose production

No large scale production of microbially derived cellulose has been attempted to date. Thus critical data needed for verification of scale up parameters are lacking. The economy of scale will most certainly determine the ultimate production price for the product, but this price cannot be accurately set until additional fermentation research is completed. Considering these limitations and the reserve of large companies to engage in potentially high risk enterprises, what are the prospects of microbially derived cellulose as a useful commodity product? The future depends on several factors, among them the commitment of major R & D support for fermentation research, the discovery of more efficient strains, and the results of fundamental research into the nature of the cellulose synthase system -- its regulation and its potential for genetic manipulation.

Optimal fermentation pathways in relation to final product characteristics

If the industrial production of microbially derived cellulose required only a deep tank fermentation of a suspended product, the fermentation engineering would be straightforward inasmuch as parameters such as pH, oxygen, viscosity, nutrient input, and batch outflow, all could be controlled with fermentation technology similar to that currently used for such industrially important polysaccharides as xanthan gum. In fact, Cetus and Weyerhaeuser have used this approach to produce a slurry of partially dried microbially derived cellulose known as "Cellulon". Current uses for this product are rather limited in view of product applications which can be adequately fulfilled by conventional sources of cellulose or cellulose derivatives.

Fermentation designed to produce intact microbial cellulose membranes, on the other hand, leads to a product with unmatched strength and water absorption properties (White and Brown,

1989). While this type of product would be more readily appreciated from the market perspective, it is difficult to predict the degree to which the cellulose yield can be perfected using this method. Nevertheless, for the present, it looks as though passive fermentation of cellulose membranes is reasonable and cost effective, provided sufficient space is available for fermentation and the space is efficiently utilized.

What would be some of the characteristics of such a passive membrane fermentor? First, it needs to have a sufficiently oxygen-permeable surface to permit optimal cellulose production. Too much or too little oxygen can be detrimental to cellulose production. Second, the fermentor needs to be automated with respect to the operations of nutrient delivery, inoculation, cellulose production, and cellulose harvesting. Third, this passive fermentor must be modular to protect against loss due to accidental contamination. All of these characteristics can be incorporated using present technology, and our laboratory is committed through a State of Texas grant to the development of fermentation technology for microbial cellulose.

Molecular biology and designer genes for cellulose production

Short term strategies will most likely inaugurate the era of intermediate and large scale fermentation of microbial cellulose; however, for this technology to be truly revolutionary, we must utilize genetic engineering (Saxena, Lin and Brown, 1990, 1991). It is here that the potential for improvement of cellulose biosynthesis is greatest; however, we need to know what to improve! We need to develop, over many years, a thorough well-integrated understanding of the *natural process of cellulose biosynthesis*. What nature has done over the millennia is to perfect adaptation to niches. If we can understand the overall process, the task for the genetic engineer becomes much easier. For example, the movement of eukaryotic cells from the sea to the land required adaptation to a less buoyant environment. This meant that extensive cell wall structural modifications were required. Along with the cellulose came the requirement for other polymers to integrate into a composite material for maximum strength at the minimal cost for the energy budget of the cell. For example, cells did not evolve using chitin as the fibrillar material in the wall, because chitin contains nitrogen, and for the equivalent structural performance, cellulose with only H, O, and C would work equally well. The evolutionary strategies were really quiet complicated when one thinks of the various biochemical pathways required to assemble a typical eukaryotic plant cell wall with its cellulose, pectin, hemicellulose, and lignin components.

So what genetic engineering strategies should we pursue? In the case of microbial cellulose production, we need to consider introduction of the cellulose synthesis operon into a photosynthetic prokaryotic cell, one which is also capable of nitrogen fixation and growth in extreme environments such as highly saline ponds. If this were successful, a number of onerous criteria could be avoided. First, the extreme environment could negate the necessity for maintenance of sterile conditions, a great potential cost saving. Second, the energy budget could be reduced significantly without need for nitrogen-based fertilizers and carbon-based substrates. Certainly, this is a theoretical consideration at this point, but it is important to define the ultimate goals which, if sought and achieved, could dramatically change the agricultural practices throughout the world.

One final thought is that a better understanding of the molecular biology and biochemistry of microbial cellulose can result in more productive and efficient cellulose from conventional sources such as cotton and trees. The earth and its inhabitants can benefit not only from an industrial transition to a microbial cellulose economy thus reducing deforestation and freeing additional arable land for food production but also from more efficient cellulose production on land because the overall CO_2 balance will continue to depend on land plants. Increasing land plant cellulose production efficiency will also help achieve the goal of providing for human needs without disastrous consequences on our environment.

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