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Lessons from the cow: What the ruminant animal can teach us about consolidated bioprocessing of cellulosic biomass

Paul J. Weimer [a,b,*], James B. Russell [a,c], Richard E. Muck [a,d]

[a] USDA-ARS-US Dairy Forage Research Center, 1925 Linden Drive West, Madison, WI 53706, United States
[b] Department of Bacteriology, University of Wisconsin-Madison, Madison, WI 53706, United States
[c] USDA-ARS – Plant, Soil and Nutrition Laboratory, Robert C. Holley Center for Agriculture and Health, Tower Road, Cornell University, Ithaca, NY 14853, United States
[d] Department of Biological Systems Engineering, University of Wisconsin-Madison, Madison, WI 53706, United States

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ABSTRACT

Consolidated bioprocessing (CBP) of cellulosic biomass is a promising source of ethanol. This process uses anaerobic bacteria, their own cellulolytic enzymes and fermentation pathways that convert the products of cellulose hydrolysis to ethanol in a single reactor. However, the engineering and economics of the process remain questionable. The ruminal fermentation is a very highly developed natural cellulose-degrading system. We propose that breakthroughs developed by cattle and other ruminant animals in cellulosic biomass conversion can guide future improvements in engineered CBP systems. These breakthroughs include, among others, an elegant and effective physical pretreatment; operation at high solids loading under non-aseptic conditions; minimal nutrient requirements beyond the plant biomass itself; efficient fermentation of nearly all plant components; efficient recovery of primary fermentation end-products; and production of useful co-products. Ruminal fermentation does not produce significant amounts of ethanol, but it produces volatile fatty acids and methane at a rapid rate. Because these alternative products have a high energy content, efforts should be made to recover these products and convert them to other organic compounds, particularly transportation fuels.

1. Introduction

Recent interest in converting cellulosic biomass to transportation fuels, particularly ethanol, has focused on two conversion platforms (Lynd et al., 2002). The most common approach has sought to couple the saccharification of plant cell walls using fungal polysaccharide hydrolase enzymes (produced in a dedicated bioreactor) with yeast or bacteria that subsequently convert the resulting sugars to ethanol in a separate bioreactor (SSF, simultaneous saccharification and fermentation). The alternative platform, consolidated bioprocessing (CBP), uses bacteria that possess enzymes to hydrolyze pretreated biomass, as well as a fermentation pathway that converts the resulting hydrolytic products to ethanol and other compounds in the same bioreactor. CBP typically results in a lower ethanol concentration and yield than the two-step approach that employs yeast, but it is an inherently simpler system that, at least in theory, involves less machinery and could eventually operate as a continuous system. These latter attributes have justified continued efforts to overcome current limitations of lower ethanol concentration and yield (Lynd et al., 2002, 2005). We propose here that improvements in CBP could be guided by, or derived from, processes that already exist in natural anaerobic systems – in particular the rumen ecosystem of ruminant animals, arguably the most elegant and highly evolved cellulose digesting system in nature (Hungate, 1950, 1966; Russell, 2002).

Through their ability to convert cellulosic biomass to milk, meat, wool and hides, ruminant animals have served mankind through many millennia and may be regarded as the foundation of animal agriculture. The underlying basis of ruminant cellulosic biomass conversion is a very large (up to 80 l) pre-gastric fermentation chamber, the rumen. Owing to the sheer abundance of domestic ruminant animals (1.522×10^9 cattle, 1.851×10^9 sheep and goats; United Nations Food and Agriculture Organization, 2004), the ruminal fermentation can be viewed as the world's largest commercial fermentation, with a net volume of some 2×10^{11} l. Wild ruminants, subsisting almost totally on a diet of cellulosic biomass, augment this global "reactor volume" even further.

The evolutionary and commercial success of the ruminal fermentation as a natural form of CBP raises some important questions regarding "engineered" CBP (hereafter designated as eCBP). How do the strategies of ruminal fermentation and eCBP compare in terms of the rate and extent of substrate utilization and product formation? Can specific operational features of the ruminal

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* Corresponding author. Address: USDA-ARS-US Dairy Forage Research Center, 1925 Linden Drive West, Madison, WI 53706, United States. Tel.: +1 (608) 890 0075; fax: +1 (608) 890 0076.
E-mail address: Paul.Weimer@ars.usda.gov (P.J. Weimer).

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fermentation guide improvements in eCBP? What limits the ruminal fermentation, and can these limitations be extrapolated to eCBP? Because ethanol is not a significant end-product of ruminal fermentation, another question then arises. Can anaerobic end-products other than ethanol be converted to compounds that could serve as transportation fuels or other industrial chemicals? We will begin with a general description of ruminant animals – particularly grazing domestic bovines – and their ruminal fermentation. Individual steps of biomass processing will then be evaluated through the lens of current or proposed processes for eCBP.

2. The ruminant animal and its feeding behavior

When grasses evolved as a dominant form of vegetation, ruminant animals co-evolved as species that could consume and digest this lignified and relatively resistant form of plant material. Ruminants comprise the suborder Ruminantia, Order Artiodactyla of Class Mammalia. There are currently more than 180 species of ruminants that range in size from dik-diks that weigh 3–6 kg, to buffalo that can weigh more than 1000 kg. Ruminants have been, and continue to be, the world's dominant herbivores. Some non-ruminant species (e.g., horses, zebras, rabbits, and rodents) also have a large fermentative capacity. However, this fermentation occurs post-gastrically (usually in the cecum), and the microbial protein produced by the fermentation can only be harvested by coprophagy. Ancient man recognized the relatively docile nature of some ruminants and their potential capacity to convert grass into food (meat and milk) and other useful products (e.g., hides and wool) (Clutton-Brock, 1999). Man's efforts in ruminant domestication were largely devoted to cattle (bovines), sheep (ovines), and goats (caprines). Camels have a digestive anatomy that differs slightly from those of "true ruminants", but their pre-gastric fermentation is remarkably similar.

After World War II, crop surpluses led to the use of cereal grain as a key ingredient in ruminant rations. However, ruminant evolution has long been geared towards cellulose digestion, and current shortages of grain could lead to a resurgence in forage feeding to ruminants. In our analysis we will focus our attention on bovines (cattle), not merely because they produce much of our meat and virtually all of our milk, but because, when maintained as grazing animals, cattle represent the evolutionary pinnacle of cellulosic biomass utilization. As noted by Van Soest (1994), in contrast to sheep, goats and most wild ruminants, cattle have a high muzzle width ratio. This ratio limits their ability to select the most digestible forms of forage. As a result, cattle on pasture are non-selective grazers, rather than selective "browsers". Moreover, their buccal anatomy, particularly their dentition pattern (quantifiable as a high "hypsodonty index", see Van Soest, 1994), facilitates grinding, tearing and "peeling" of highly fibrous feeds. The end result is a potentially greater intake of forage fiber than in other ruminants.

3. Follow that feed

We can next consider what happens to plant biomass during its journey through the bovine digestive tract. Where possible, we will compare the steps of the process to the transformations in a cellulosic biomass biorefinery.

3.1. Intake

Ruminant intake is largely governed by "rumen fill", which provides a satiety signal to the cow (Mertens, 1997). In the dairy cow, intake regulation establishes a range of ruminal solids contents of 12–18% on a dry matter (DM) basis. Because diets rich in forage have a low energy density and are typically digested more slowly than are cereal grains, modern cattle producers have increasingly substituted cereal grains for forage. However, this substitution forces producers to walk a fine line between maximizing production and avoiding disease problems (rumen acidosis, fatty liver, diarrhea, or laminitis) that result directly or indirectly from insufficient levels of forage fiber in the diet (Mertens, 1997). Most of our data on the feed intake of cattle are based on diets that combine cereal grain and forage. However, for our model of the rumen as a cellulosic biomass refinery, it is more useful to consider only grazing cattle not fed grain or other supplements. In the discussion below, we shall consider, as our standard of comparison, a dairy cow consuming 20 kg (DM basis) of forage per day.

3.2. Pretreatment

Proposed eCBP systems envision chemical pretreatment to make the cellulose component of the biomass more susceptible to enzymatic hydrolysis. Numerous pretreatment strategies have been evaluated (see the excellent perspective by Wyman et al. (2005)). Chemical pretreatments are effective in reducing the protective effect of lignin within the plant cell wall matrix, and the better pretreatments can produce cellulose that is almost completely degradable by cellulases, at least at laboratory scale with high enzyme loadings (Spindler et al., 1989). However, all chemical pretreatments have one or more disadvantages. These include substantial costs for reagent, the requirement for heating or pressurization of the pretreatment vessel, as well as capital costs for the pretreatment system itself. Chemical pretreatments also result in some loss of fermentable carbohydrate as well as production of other chemicals that can inhibit downstream enzymatic hydrolysis and/or microbial fermentation. Once the cellulose has been pretreated, the waste must then be handled in an economical and environmentally acceptable manner.

In ruminants, biomass pretreatment occurs purely by physical (as opposed to chemical) means. The physical processing is accomplished by initial chewing (mastication) of the feed immediately after intake, and rumination of partially regurgitated, fully hydrated feed between meals (the so-called "chewing of the cud"). The magnitude of the particle size reduction, due primarily to rumination, is truly amazing. Only particles less than 2 mm in size can readily pass from the rumen through the omasum, the organ directly downstream of the rumen that acts a filtering device. Microscopic observation of rumen contents typically reveals an abundance of feed particles that are smaller than ruminal protozoa and not much larger than ruminal bacteria. Simple calculations indicate that the increase in forage particle surface areas can be as great as 10^4-fold, and this increase in surface area is the key factor regulating the rate of cellulose fermentation in the rumen (see following section). When the forage becomes highly lignified and "woody," the ability of ruminants to reduce the particle size of ingested feed declines and so does the fermentation rate. Cows can consume poor quality forages (e.g., mature switchgrass) and by-products of the human food industry that would otherwise be wasted, although they have not mastered eating trees.

Mechanical grinding of biomass in a biorefinery has generally been considered impractical and too expensive as a pretreatment strategy. As noted in the previous section, the cow does this routinely. So just how much effort is required of the animal for this process? Grazing cattle typically will take $\sim 3 \times 10^4$ bites per day in eating alone (Table 1), and in addition will engage in ~500 rumination bouts (Mackie, 2002). Mertens (1997) reviewed numerous studies in which cows were fitted with chewing monitors. Based on these studies, the amount of time cows fed long hay devoted to total chewing (eating and ruminating) was typically in the range of 100–200 min/kg fiber intake/d, or approximately 50–150 min/kg DM/d. Cows fed mixed rations containing grains and other concen-