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MINI-REVIEW

Solvent production from xylose

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Abstract

Xylose is the second most abundant sugar derived from lignocellulose; it is considered less desirable than glucose for fermentation, and strategies that specifically increase xylose utilization in wild type or engineered cells are goals for biofuel production. Issues arise with xylose utilization because of carbohydrate catabolite repression, which is the preferential utilization of glucose relative to xylose in fermentations with both pure and mixed cultures. Taken together the low substrate utilization rates and solvent yields with xylose compared to glucose, many industrial fermentations ignore the xylolytic portion of the reaction in lieu of methods to maintain high glucose. This is shortsighted given the massive potential for xylose generation from a number of sustainable biomass feedstocks, based on utilization of the hemicellulose fraction(s) that enter pretreatment. A number of strategies have been developed in recent years to address xylose utilization and solvent production from xylose in systems with just xylose, or in systems with mixtures of glucose plus xylose, which are more typical of pretreated lignocellulose. The approaches vary in terms of complexity, stability, and ease of introduction to existing fermentation infrastructure (i.e., so-called drop-in fermentation strategies). Some approaches can be considered traditional engineering approaches (e.g., change the reaction conditions), while others are more subtle cellular approaches to eliminate the impacts of catabolite repression. Finally, genetic engineering has been used to increase xylose utilization, although this can be considered a relatively nascent approach compared to manipulations completed to date for glucose utilization.

Keywords Biofuels · Bio butanol · Xylose fermentation

Introduction

Solvents such as butanol, ethanol, and acetone are targets for both biofuels as well as biologically synthesized precursors in fine chemical production (Szwaja and Naber 2010; Cooney et al. 2009; Masum et al. 2014). Bio-butanol (technically *n*-butanol, but hereafter referred to as simply butanol) is particularly attractive as a supplement for gasoline-powered transportation infrastructure due to its combustion properties and relatively high energy content when compared to ethanol. One impediment to advancing bio-butanol as a global fuel is the lack of reasonable lignocellulosic feedstocks that can be fermented efficiently. Solvent production is generally divided into prokaryotic processes (e.g., *Clostridia* fermentations), and eukaryotic processes, which is mostly glucose and xylose fermenting yeasts. The following review only addresses advances in prokaryotic processes.

Butanol is generally considered an alternative to ethanol as a biologically derived liquid fuel, and the market is expected to expand through 2021 (de Maria 2016; Harvey and Meylemans 2011). The energy content of butanol is higher than ethanol; butanol releases 29.2 MJ L^{-1}, and ethanol releases 19.6 MJ L^{-1} (Lee et al. 2008). As previously happened with methyl tert butyl ether (MTBE), the petrochemical industry has coalesced around butanol because it is more stable in storage and transport, and blends more readily with refined fuel. Biological processes such as acetone-butanol-ethanol (ABE) fermentation from lignocellulosic biomass are being researched to supplement or replace fossil fuels (Jiang et al. 2009; Atsumi et al. 2008; Connor and Liao 2009; Lee et al. 2012). Figure 1 is a conceptual model of the critical pathways in ABE fermentation, which indicates that multiple products can be generated during single fermentations.

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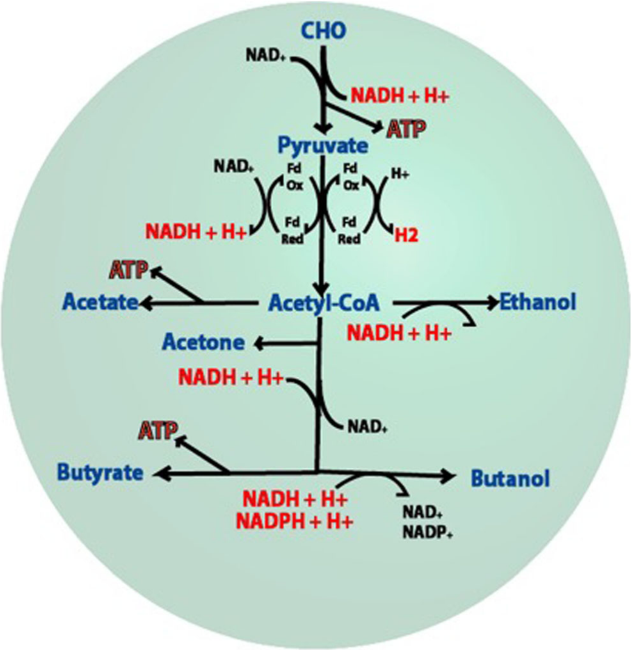

Fig. 1 Conceptual model of carbohydrate fermentation leading to acetone, butanol, and ethanol during ABE fermentation

ABE fermentation has been studied for almost a century, and the technology has been refined at both the cellular and engineering (bioreactor) scales. While the technology has always held promise, it does suffer from low solvent yields, limited substrate utilization, and low biomass turnover rate (Green 2011). Research addressing these limitations has focused on genetic modifications and/or reactor design modifications to increase solvent output, rather than specific yield (Harris et al. 2001; Qureshi and Maddox 1988; Roffler et al. 1987). One major issue is that solvent producers are seeking so-called drop-in technologies, which work with existing reactor infrastructure without too many modifications (Liang et al. 2002; Alkasrawi et al. 2003). While this is a very good goal, it is difficult in practice to adapt full-scale fermentations without changing the actual reactors involved.

Hemicellulose monomers, the most critical of which is xylose, are desirable feedstocks for industrial ABE production since they do not compete directly with human food sources (Valentine et al. 2012). Xylose, a pentose sugar, comprises 30% of all plant-derived biomass (Kumar et al. 2009). The primary issue is that many microorganisms cannot ferment xylose effectively, or they lack the necessary machinery to transport and assimilate it into central metabolism (Gírio et al. 2010). This has made glucose the preferential sugar substrate, since it is readily fermented by most ABE producing strains (El Kanouni et al. 1998). Figure 2 indicates the general path from lignocellulosic biomass, to xylose-derived products.

Members of the genus *Clostridium* rely either on xylose proton symporters or ATP-dependent xylose transport proteins to transport xylose across the cell membrane (Servinsky et al. 2010). Xylose is transformed prior to glycolysis, requiring ATP hydrolysis and regeneration of NAD^+ and $NADP^+$ cofactors (Jeffries 1983). Glucose is typically 100% fermented by the cells of interest, while xylose in ABE fermentation can be fermented at 50% or less (Xiao et al. 2012; Wu et al. 2016b; Mes-Hartree and Saddler 1982).

Solvent production with glucose versus xylose

Much attention has been placed on replacing glucose as a feedstock for current butanol fermentations overall substrate cost and competition with food sources (Yang et al. 2015; Xue et al. 2013; Zhang 2011). Recently, many have been investigating the use of hemicellulose as a feedstock due to natural abundance. Hemicellulose is a polymer which composes nearly 30–40% of the earth's carbon (Kumar et al. 2009; Gong et al. 1981; Xiao et al. 2011), and xylose and arabinose are pentose monomers which compose hemicellulose. Although terrestrially abundant, pentose catabolism is limited or nonexistent in many industrially relevant organisms, and although xylose flux is poorly understood in solventogenic organisms, many efforts are being made to elucidate the processes surrounding its uptake and metabolism for manipulation.

The favorability of hexose over pentose sugars is apparent from the rate of substrate co-fermentation within *Clostridia* (Chen et al. 2013; Grimmler et al. 2010; Jeffries 1983). Xylose can be metabolized in bacterial cells following entry catalyzed by proton motive force (symport) (Jeffries 1983; Walmsley et al. 1998; Ma 1958). Following entry into the cell, xylose is metabolized via the pentose phosphate pathway (PPP) or the phosphoketolase pathway in organisms such as *C. acetobutylicum*, accounting for the slower rate of utilization attributed to the requirement of additional metabolic steps prior to entry into central and/or solventogenic downstream pathways.

In the PPP, xylose is phosphorylated to xylulose-5-phosphate, and from this position, xylulose-5-phosphate is converted to fructose-6-phosphate or glyceraldehyde-3-phosphate, allowing for entry into glycolysis (Ma 1958). Recently, evidence has shown that under high xylose concentrations ($20\ g\ L^{-1}$), the phosphoketolase pathway in *C. acetobutylicum* is upregulated, indicating that some solventogenic *Clostridia* possess the ability to metabolize xylose in parallel pathways under high substrate stress (Liu et al. 2012). This allows for xylose to be converted to glyceraldeyhyde-3-phosphate or acetyl-phosphate, which can be shunted to acetyl-CoA, thus bypassing glycolysis if the phosphoketolase pathway is activated.

Reports suggest that it is this difference in substrate uptake that may be responsible for the preferential utilization of glucose by solventogenic microbial genera. It makes sense from

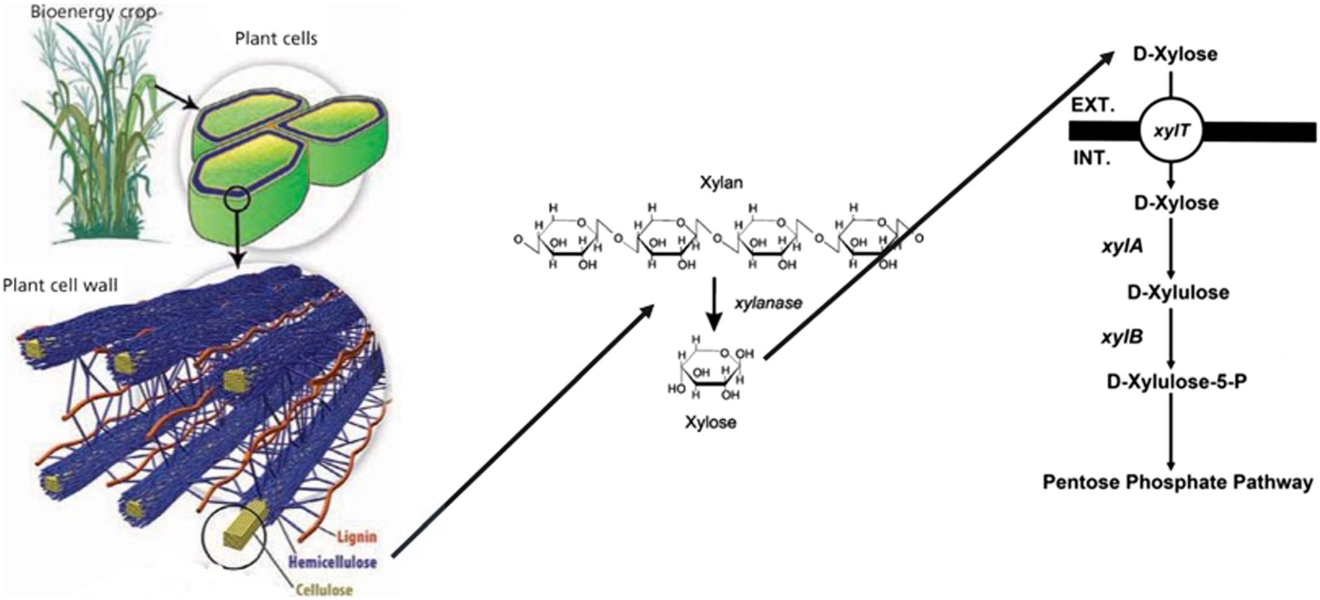

Fig. 2 Diagram depicting xylose extraction from the hemicellulose portion of feedstock biomass, and its subsequent conversion in biological reactions

an energy balance perspective; xylose uptake is active and consumes ATP, while glucose uptake is passive and does not necessarily require ATP.

Carbohydrate catabolite repression (CCR) assures that glucose will be preferentially utilized and that xylose will not be taken up by the cells until glucose falls below a threshold concentration. CCR is a signal cascade in cells that can utilize both glucose and xylose, in which metabolites of the glucose uptake and utilization pathway downregulate genes involved in xylose utilization. This mechanism decreases the value of high xylose content lignocellulosic biomass. If cells are going to preferentially utilize glucose, and the solvent yield from xylose is generally low, then most industrial fermentations are going to ignore the xylose fermentation component altogether and focus only on glucose. Again, this is a very limiting approach given the mass of xylose that can be developed from many lignocellulosic sources. A number of processes can help overcome this *xylose limitation*; they are introduced below.

Acetone, butanol, ethanol (ABE) fermentation with xylose as the primary substrate

The methods tested for increasing xylose utilization for solventogenic fermentations have generally fallen within three broad areas: (a) developing mixed microbial cultures that will increase overall xylose utilization kinetics, and therefore the solvent generation kinetics, (b) amending continuous or fed-batch reactors with electron transfer molecules that disrupt overall metabolism and change substrate utilization rate and extent, and (c) genetic engineering of specific xylose uptake and utilization genes for increased solvent production. These methods can also be combined to maximize xylose utilization and solvent yield. Each of these is discussed below.

Co-cultures and mixed cultures

Pure cultures are generally more desirable with which to work during industrial fermentations, mainly because of purity of the products generated and the absence of cells that may metabolize the end products. In addition, the fermentation conditions are simpler when only a single genus or species is present; multiple cells may have diverse nutritional needs. This has worked very well for glucose fermentation, but less so for xylose fermentation.

Developing mixed cultures or co-cultures with two specific microbial genera has also been a long-standing method of generating desirable end products. Co- and mixed cultures are sometimes more stable than pure cultures, which means that they can be grown in continuous culture as opposed to fed batch mode, which slows production. Co- and mixed cultures also respond differently to metabolites; there may be less repression due to one member of the community quickly utilizing glucose which allows the xylose fermenter to directly ferment xylose even in reactors with both substrates present.

A recent co-culture was developed using a yeast, *Saccharomyces cerevisiae*, and *Clostridium acetobutylicum*, which fermented mixed glucose plus xylose and generated high solvent yields when both cells were present (Qi et al. 2015). Initially, it was thought that pre-treating the substrate mixture with *S. cerevisiae* would be the most effective method of increasing overall solvent yield, with the yeast quickly eliminating glucose from the medium and allowing *C. acetobutylicum* to ferment the xylose unaffected by CCR. Solvent yield in this case was 0.03 g g^{-1} higher than the non-pre-treated substrate mixture. However, when cells were incubated together rather than in series, the overall productivity rates (g L^{-1} h^{-1} solvent generated) doubled, with simultaneous glucose and xylose utilization. While successful, this method does require specialized culture conditions that are favorable to both the yeast and the Bacterium. It is unknown if the dual-favorable conditions decrease overall productivity, given that the fermentation conditions are not ideal for either cell involved.

A co-culture developed using *C. beijerinckii* and the Fe^{3+} reducer *Geobacter metallireducens* increased xylose utilization and subsequent hydrogen and solvent production (Zhang et al. 2013a, Zhang et al. 2013b). Glucose utilization was the same amongst all treatments with this co-culture, and both the glucose utilization rate and solvent yields were similar in the co-culture and the pure culture *C. beijerinckii*. However, xylose utilization increased in the co-culture, especially when inoculated with an electron shuttling molecule (anthraquinone 2,6-disulfonate, AQDS) that serves as an electron acceptor for *G. metallireducens*. The suggested mechanism was that acetate utilization by the respiratory *G. metallireducens* altered substrate uptake kinetics, possibly by limiting catabolite repression or changing the overall energy balance of the combined reactions. *Geobacter* are not butanol oxidizers, so this system was suggested as a potential butanol generating industrial co-culture.

Most recently, a genetically engineered co-culture of *Escherichia coli* was developed that limited the impacts of CCR on the cells and increased the overall rate and extent of xylose fermentation to generate butanol (Saini et al. 2017). An *E. coli* strain was isolated that was not sensitive to the impacts of CCR and could therefore utilize xylose and glucose without specific preference. However, xylose-derived butanol yields remained low. The co-culture distributed the necessary reactions between two different strains, which increased overall butanol yield. The primary mechanism segregated butyrate synthesis and butyrate conversion between the glycolytic strain and the xylolytic strain, with the glycolytic strain converting the majority of the butyrate to butanol. In addition, eliminating the glucose-6-phosphate dehydrogenase pathway prevented glucose metabolism from directly impacting xylose metabolism, by limiting glucose interaction with the pentose phosphate pathway. This allowed simultaneous glucose and xylose fermentation, without glucose catabolism interfering with the xylose fermentation pathway. However, as with many genetically modified or even discovered mutant strains, these reactions were first predicated on having obtained the CCR-insensitive strain (Saini et al. 2017).

Batch and continuous fermentation amendments or enhancements

Technologies or strategies that merely amend existing pure, co-, and mixed cultures are sometimes considered ideal from a practical perspective. These are the so-called drop in strategies that require little or no change to existing infrastructure and also do not require the presence of sensitive, costly, or fastidious microbial cultures. They are often considered a replacement for genetic modification, but amendments to actual culture conditions can be used with any cells, whether the cells are wild-type, mutant, or genetically modified strains.

The simplest manipulation is pH, which impacts the onset and longevity of solvent production during ABE fermentations. Acids generated lower the pH, and each cell has a pH threshold at which acidogenesis slows down and solventogenesis increases. Several data suggest that xylose utilization increases, primarily in pure cultures, when pH is controlled and is maintained slightly higher than would be expected if the fermentation was allowed to go to completion, with the pH dropping to 4.0–4.5 (Jiang et al. 2014; Procentese et al. 2015). However, the impacts of pH seem to be most profound xylose is alone as the fermentable substrate. Jiang et al. (2014) report that xylose utilization and solvent production productivity and yield doubled, when the pH was maintained at 5.0 as opposed to dropping below 4.0 over the course of a 100-h fed-batch reaction with *C. acetobutylicum* (Jiang et al. 2014). Cell yield was significantly higher in the controlled pH reactor, which may have been partially responsible for the increases reported. The results were less pronounced when glucose and xylose were simultaneously fed to the reactor, and systems with higher glucose/xylose ratios were not influenced by pH; the yields were similar irrespective of pH control (Jiang et al. 2014).

Qiu et al. (2016) demonstrated that a municipal sludge derived mixed culture generated hydrogen and solvents with xylose as the sole substrate at a pH range from 4.0 to 10.0 as the initial pH, but without further pH control over the course of a fed-batch reaction (Qiu et al. 2016). Phylogenetic data using 16S rRNA gene indicated that the dominant populations were expected genera including *Clostridium*, Bacteroidetes, and uncultured phylotypes most closely related to known ABE fermenters (Qiu et al. 2016). Respiratory populations were low or absent, which was expected with the lack of a terminal electron acceptor. Xylose was completely consumed in all incubations run between pH 6.0 and 9.0 as the starting pH, but utilization dropped off sharply at pH 5.0 and pH 10.0, as did all other metabolites. Hydrogen yield was relatively high in this culture in comparison to solvents, which argues that mixed cultures are less efficient at solvent production and that pH has little impact on mixed culture solvent yield.

Nutrient supplementation is another simple approach to increase xylose fermentation and solvent yield, and data suggest that zinc impacts xylose-derived ABE synthesis. *Clostridium acetobutylicum* was incubated in several volumetric scales of batch reactor with xylose as the sole fermentable substrate or in mixtures with glucose, and zinc added at 1.0 mg L^{-1} (which was operationally defined as high relative to the basal medium). High zinc concentrations increased xylose utilization and solvent production, and the data indicated that perhaps zinc impacted xylose transport, but at this point, the exact mechanism is unknown (Wu et al. 2016a, Wu et al. 2016b). The data indicate a modest synergistic effect of co-added calcium at higher than basal level concentrations and that the combined nutrients influenced carbon flux within the cells from predominately acidogenesis to predominately solventogenesis. They attributed this to an uncharacterized "redox balance" (Wu et al. 2016a, Wu et al. 2016b), which leads to the last drop in manipulation that can be performed.

Fermentation in general is predicated on redox balance; the absence of a terminal electron acceptor necessitates a greater balance between inputs, metabolites, and outputs (products) during fermentation. Anything that unbalances the fermentation process increases or decreases the rate at which substrates are fermented, and the productivity and yield of solvents generated.

Redox active molecules and electron shuttling compounds (e.g., neutral red or anthraquinone-2,6-disulfonate (AQDS)) are reported to alter fermentative metabolism, although the exact mechanisms involved are not yet known. Thus far, all data suggest that redox mediators increase solvent production rates, and overall solvent titers; however, most data were generated with glucose as the sole fermentable substrate (Girbal et al. 1995; Peguin et al. 1994a, Peguin et al. 1994b; Peguin and Soucaille 1996; Vasconcelos et al. 1994). The first reports demonstrated that electron mediators such as neutral red and methyl viologen altered substrate oxidation, solvent production, and more recently hydrogen production (Park et al. 1999; Peguin and Soucaille 1995; Rao and Mutharasan 1987; Ye et al. 2012). Solvent production was generally influenced by the redox mediator methyl viologen, in pH-controlled fed-batch reactors with different *Clostridial* cultures; however, the single most affected variable was butanol production, and subsequent studies focused on this strategy for increasing butanol output (Peguin and Soucaille 1996). Most recently, a series of fed-batch reactors were manipulated using a potentiostat to poise the aqueous medium at operationally defined "near oxic" conditions, and data indicated that solvent yield increased relative to controls (Shin et al. 2002). It is unsurprising that fermentative redox manipulations went in this direction, given the increase in electrode manipulations for microbial reactions over the past several years. Although no exact mechanisms were developed for any of these systems, the best theory was that NAD^+/NADH ratios were altered and that in turn increased production of solvents by redirecting carbon and electrons to these pathways (Meyer and Papoutsakis 1989; Singh et al. 2009). Again, in all cases referenced above, glucose was the sole substrate and experiments did not address improving glucose utilization due to extracellular electron transfer. More recent data have considered substrate utilization, and substrates other than glucose.

While early studies did not consider impacts on xylose utilization, they were the foundation upon which the current field of unbalanced fermentation has been built, for the purpose of increasing xylose utilization and solvent recovery. The term unbalanced fermentation refers to any strategies or technologies that introduce an extracellular electron acceptor that is not used in respiratory processes to generate energy as adenosine triphosphate (ATP). In other words, a so-called electron sink is added to fermentative cultures, which redirects some fraction of electron flow. The general result is a disruption of standard fermentation, which expresses itself in different ways.

Exogenous molecules are not even necessary to increase butanol production from sugars, just merely a change in redox potential. Recent reports suggest that fermentations conducted in the presence of electrodes that maintain "oxidizing" conditions (E_H between +0.2 V and + 0.4 V) increased butanol production (Wietzke and Bahl 2012). Enzyme data suggest that the cells (*Clostridium acetobutylicum*) expressed a redox sensing protein (Rex), which is still being characterized (Wietzke and Bahl 2012). Xylose utilization was not part of that specific study, but the researchers suggested that this change would impact all fermentable substrates.

Hydrogen is the functional electron sink for fermentation. Excess reducing equivalents are expended during rapid fermentation as molecular hydrogen, which is released extracellularly to maintain an intracellular redox balance (Xin et al. 2014; Ye et al. 2011, Ye et al. 2012). Fermentative cells, however, will transfer electrons to a number of molecules which includes redox active quinones (e.g., AQDS), ferric iron, and manganese dioxide (Popovic and Finneran 2018; Popovic et al. 2017; Ye et al. 2011, Ye et al. 2012; Zhang et al. 2013b). These electron sinks alter carbon and electron flow during fermentation, which simultaneously increases xylose utilization and solvent production. In some cases, the overall biomass yield increases, but these are not being used as respiratory terminal electron acceptors (Popovic et al. 2017 #3525).

Fed batch reactors of *C. beijerinckii* amended with either AQDS or riboflavin were incubated with and without ferric iron (which was added as the common environmental mineral phase ferrihydrite). Data demonstrated that extracellular electron transfer disrupted metabolism similarly to prior molecules, but in this case, the ultimate electron sink was the environmentally relevant mineral iron, which has implications for natural systems as well as engineered systems. The process by which redox active molecules accept and then donate electrons to an alternate acceptor is referred to as electron shuttling (Popovic et al. 2017), and has been used in environmental remediation for several decades. It is only now gaining momentum in engineered reactors.

The changes to xylose utilization rate and extent, as well as the butanol production yields, in any system amended with both electron shuttling molecules and ferric iron were substantial. It was especially notable because these cultures were not incubated with acetate, which is added to increase butanol yield in traditional fermentations. Past data indicated that electron shuttles alone increased butanol yield, but the continuous recycling of the oxidized form of the molecule (by electron transfer to ferric iron) increased xylose utilization as well. The redox mediators did not impact glucose; it was always utilized 100% irrespective of the test conditions. However, extracellular electron transfer did increase butanol production with glucose as the primary substrate (Popovic et al. 2017; Zhao et al. 2017). These data demonstrate a strategy for targeting xylose uptake and utilization in solvent generating wild-type cells, which could be retrofitted to existing fermentation systems without the need to radically alter engineering infrastructure (and therefore move in the direction of a "drop in" technology).

An electron flow conceptual model was developed to describe the fate of reducing equivalents and carbon in ABE fermentations that were subjected to unbalanced fermentations, and how carbon and electron flow amongst the various pathways responsible resulted in increased butanol production. Data suggest that increased ATP yield and NAD+/NADH ratios are critical to both (Popovic et al. 2017; Zhao et al. 2017), but more work is required to elucidate the exact mechanisms by which redox active quinones and/or ferric iron increase xylose utilization and increased butanol production.

Genetic engineering of known xylose fermenters

Genetic engineering can include insertion or deletion of specific genes that impact xylose utilization and solvent production, or genetic tools to develop mutants that selectively utilize xylose to generate solvents. The majority of recent genetic advances have focused on two metabolic pathways: xylose uptake and phosphorylation, or altering the carbon catabolite repression system (Basu et al. 2017; Boonsombuti et al. 2014; Bruder et al. 2015; Liu et al. 2018; Ratnaparkhe et al. 2016; Zhang et al. 2017). Table 1 summarizes the reported genetic modifications and/or mutant strains that have been developed in support of xylose fermentation for solvent production.

Lignocellulosic pretreatment for xylose recovery and butanol production

Feedstock pretreatment is a critical aspect of solvent production. While past pretreatment approaches have selected against xylose enrichment, a number of recent pretreatment advances specifically select for xylose. Pretreatment is an entirely different subject, but it is worth mentioning here that several different lignocellulosic materials can be treated to produce high masses of xylose and that xylose will be available for ABE fermentation.

Materials that have been reported to date include birch Kraft black liquor, enzyme-treated corn fiber hydrolysate,

Table 1 Genetic modifications or analyses for xylose-mediated solvent production

Wild-type cell (genus/species)	Genetic modification and phenotype expressed	Impact on xylose fermentation or solvent production	Reference
E. coli	Deletion of *glk* and *xylA* genes Transformation of CCR insensitive strain to be either glucose selective or xylose selective	Increase in n-butanol production from 2.6 to 5.2 g L^{-1}	(Saini et al. 2017)
C. acetobutylicum	Development of a CCR insensitive mutant by identifying a short nucleotide catabolite responsive element, that impacted transcriptional control of CCR genes	No direct measurement of xylose utilization or solvent production, but did report a 37% increase in β-galactosidase activity	(Bruder et al. 2015)
C. tyrobutyricum	Overexpression of *xylT*, *xylA*, and *xylB*	Reported *n*-butanol titers of 15.7 g L^{-1} and *n*-butanol yield of 0.24 g g^{-1}	(Yu et al. 2015)
Propionibacterium freudenreichii	Overexpression of *xylT*, *xylA*, and *xylB*	Increased xylose consumption from less than 1 to over 18 g L^{-1}	(Wei et al. 2016)
C. tyrobutyricum	Overexpression of *xylT*, *xylA*, and *xylB*	Increased xylose utilization rates from 0.16 to 1.28 g L^{-1} h^{-1}	(Fu et al. 2017a; Fu et al. 2017b)
Clostridium strain B0H3	Proteomic profiling to identify specific xylose utilization genes up and down regulated under different growth conditions	N/A	(Basu et al. 2017)
Clostridium beijerinckii NCIMB 8052	Increasing phenolic compound tolerance	N/A	(Liu et al. 2018)
Clostridium beijerinckii NCIMB 8052	Overexpression of *adhE2* and *ctfAB*	Increased ABE production from mixed sugar substrates derived from sugarcane bagasse	(Lu et al. 2017)

acid-treated corn fiber, sea weed extract, and wood pulp hydrolysate (acid treated) (Kudahettige-Nilsson et al. 2015). Data from studies done with these varying materials suggest that ABE yield differs depending on the starting biomass feedstock, but all materials generated solvents, with butanol as the solvent recovered in the highest yield for all cases reported (Kudahettige-Nilsson et al. 2015). In summary, the data suggest that butanol is the optimal solvent target for lignocellulosic biomass that is high in xylose content.

Conclusions and prospects

Xylose utilization in solvent production has increased during the past decade, and advances in research guarantee that it will continue to increase in the years ahead. All recent data suggest that the underlying questions are being investigated to develop xylose-derived solvents, primarily butanol, into a competitive market on the world biofuel stage. Lignocellulosic biomass pre-treatment technologies have become more cost effective, and the number of available feedstocks has increased. Xylose has been historically considered a low value product during pre-treatment, but that was the result of having few downstream reactions predicated on xylose use. The review above suggests that the number of available technologies for xylose fermentation to generate solvents is already modest to large, and that with each new advance, the field comes closer to having xylose be as preferable a substrate as glucose, although there is still a large gap between xylose use and glucose use.

While this has the greatest number of technological implications for biofuel production, it does raise questions as to how xylose fermentation in natural environments or alternative engineered environments (e.g., wastewater treatment) influences carbon and electron flow. The Firmicutes are often dominant members of the microbial community in all environments and therefore exert a large level of influence on carbon flow. The reactions above (i.e., biofuels) are viewed through the aperture of "products." However, if these reactions also occur in natural or non-biofuel systems, then fermentative *Clostridia* and other solvent generating genera can be viewed as influencing the remainder of the reactions, by producing molecules that serve as electron donors for respiratory cells. It is reasonable to expect that in higher Fe(III) environments, xylose fermentation will increase, and butanol will become a more prevalent product. That will select for different downstream metabolic processes than standard fermentations.

In terms of engineered biofuel production, one very active research area is the development of mixed microbial cultures rather than relying strictly on pure cultures. Mixed microbial cultures (be it binary cultures or those containing multiple different genera) are considered more stable than pure cultures. However, there is less chance of genetic manipulations, and product yields are lower. So, it is a tradeoff between stability and longevity of the culture (and the general ease of manipulating it in a reactor), versus the purity and yield of products. A number of recent studies have focused on how mixed cultures can be developed, that catalyze similar reactions, and reach similar product yields (Li et al. 2017; Panitz et al. 2014; Popovic et al. 2017; Raganati et al. 2014; Ratnaparkhe et al. 2016; Roth and Tippkotter 2016; Sandoval-Espinola et al. 2015; Van Hecke et al. 2016; Zhang et al. 2017).

One additional thought is the intersection between the redox manipulations described above, and the burgeoning field of microbial bioelectrochemistry. Bioelectrochemistry is the use of electrodes to manipulate electron donors, electron acceptors, or general redox conditions within microbial incubations. It is not unreasonable to postulate that electrodes could be used in place of redox active molecules. If successful, it is also reasonable to conclude that electrodes could be very simply added to any industrial fermentation to manipulate reactor conditions, which favor both xylose utilization and butanol generation.

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Compliance with ethical standards

Conflict of interest All authors declare no conflict(s) of interest. This article does not contain any studies with human participants or animals performed by any of the authors.

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