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Glucose and sucrose: hazardous fast-food for industrial yeast?

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Yeast cells often encounter a mixture of different carbohydrates in industrial processes. However, glucose and sucrose are always consumed first. The presence of these sugars causes repression of gluconeogenesis, the glyoxylate cycle, respiration and the uptake of less-preferred carbohydrates. Glucose and sucrose also trigger unexpected, hormone-like effects, including the activation of cellular growth, the mobilization of storage compounds and the diminution of cellular stress resistance. In an industrial context, these effects lead to several yeast-related problems, such as slow or incomplete fermentation, 'off flavors' and poor maintenance of yeast vitality. Recent studies indicate that the use of mutants with altered responses to carbohydrates can significantly increase productivity. Alternatively, avoiding unnecessary exposure to glucose and sucrose could also improve the performance of industrial yeasts.

Unlike most mammalian cells, which function in an environment with virtually constant concentrations of only one sugar, namely glucose, yeast cells encounter variable concentrations of many different carbon sources. To overcome this lack of homeostasis and to survive in a highly competitive ecosystem, the common brewer's and baker's yeast *Saccharomyces cerevisiae* has evolved the capacity to take up and to metabolize different carbohydrates. *S. cerevisiae* cells also have several mechanisms for sensing the nutritional status of the environment, enabling them to adapt their uptake and metabolism of nutrients to specific conditions. Although these signaling pathways have been studied mostly as a model for nutrient-induced signaling cascades in higher eukaryotes, their unraveling has relevance for several applications of yeast biotechnology, including alcoholic fermentation, bread production and the fabrication of biopharmaceuticals.

In this review, we begin by summarizing the role of the two best-known glucose-triggered signaling cascades in *S. cerevisiae*: the main glucose repression pathway (also known as the catabolite repression pathway), and the Ras/cAMP/protein kinase A (PKA) pathway. We then consider the role and implications of sugar signaling in industrial yeast-based processes.

Glucose regulation of carbohydrate uptake and metabolism

In response to glucose and sucrose, the main glucose repression pathway downregulates several genes involved in the uptake and metabolism of alternative carbohydrates, as well as genes involved in gluconeogenesis and respiration. The repression of respiration by glucose and sucrose, which is known as the 'Crabtree effect', might seem counterproductive but, although respiration is a more efficient method of energy production, fermentation offers the advantage of ethanol production, which hampers the growth of competing microorganisms. In addition, glucose concentrations, and possibly levels of glycolytic intermediates, regulate the expression of several glucose transporters and some glycolytic genes (Figure 1 and reviewed in Refs [1–4]). Thus, the main glucose repression pathway ensures that the preferred sugars are metabolized before the consumption of alternative carbohydrates, such as maltose and galactose.

Glucose also slows down the uptake of fructose because both sugars are imported by the same carriers, which have a greater affinity for glucose than for fructose. Besides this competitive inhibition of fructose uptake, recent research shows that glucose can repress the expression of specific fructose transporters such as

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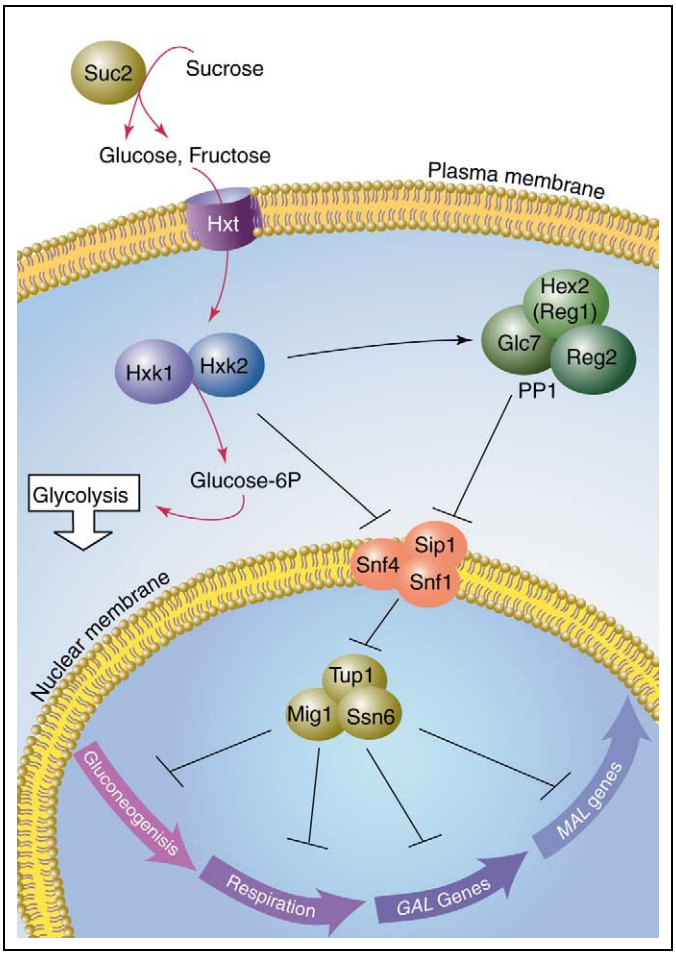

Figure 1. The main glucose repression pathway in *Saccharomyces cerevisiae*. Extracellular glucose is taken up through one of the hexose transporters (Hxt) and subsequently phosphorylated to glucose 6-phosphate (glucose-6P) by one of the hexokinases (Hxk). This phosphorylation process and/or the depletion of AMP owing to the increased production of ATP inactivates the central protein kinase Snf1 [59]. Inactivation of Snf1 occurs via either inhibition of its phosphorylation or stimulation of the Snf1-dephosphorylation activity of the phosphatase complex PP1 [60,61]. When Snf1 is inactive, the DNA-binding protein Mig1 is translocated from the cytoplasm to the nucleus [62]. In the nucleus, Mig1 recruits the general repressors Tup1 and Ssn6 and binds to the promoters of several glucose-repressed genes, including genes involved in gluconeogenesis, respiration and the uptake and breakdown of alternative carbon sources, such as maltose (*MAL* genes) and galactose (*GAL* genes). When extracellular glucose is depleted, the Snf1 complex is activated, causing the translocation of Mig1 back to the cytoplasm, where it can no longer repress its targets [62,63]. Thus, glucose repression is relieved and alternative carbon sources can be taken up. Fructose and sucrose, which is extracellularly hydrolyzed into glucose and fructose, exert catabolite repression effects similar to those of glucose, although glucose is taken up preferentially [1]. For details, see reviews by Gancedo [1], Johnston [2], Carlson [3] and Winderickx *et al.* [4].

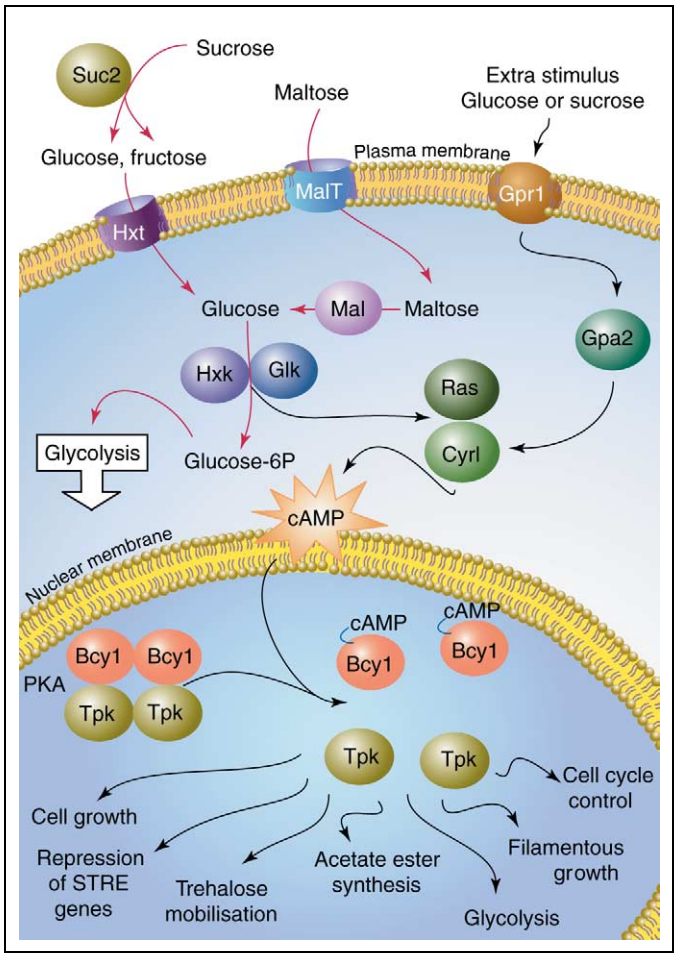

Figure 2. The Ras/cAMP/protein kinase A (PKA) nutrient signaling pathway in *Saccharomyces cerevisiae*. Full activation of this pathway requires a dual signal. First, the intracellular phosphorylation of glucose to glucose 6-phopshate (glucose-6P) enhances the activity of adenylate cyclase Cyr1. Second, extracellular glucose or sucrose is sensed by a G-protein-coupled receptor system, consisting of the receptor Gpr1 and the Gα protein Gpa2 [10]. This second signal strongly enhances Cyr1 activity, resulting in a transient peak of cAMP immediately after the addition of glucose or sucrose [9,10,57,64]. The rise in cAMP causes the regulatory subunits (Bcy1) of the PKA complex to bind cAMP and to dissociate from the Tpk catalytic subunits of PKA [65,66]. The free Tpk kinases then phosphorylate various target proteins, which eventually leads to acclimatization to high glucose levels (Figure 3). Although the role of the small G proteins Ras1 and Ras2 in this process is not completely understood, these proteins are essential for basal Cyr1 activity [67,68]. For details, see reviews by Johnston [2] and Winderickx *et al.* [4]. For clarity, note that Gpa2 and Cyr1 are not depicted as attached to the plasma membrane and the shuttling of the free PKA catalytic subunits out of the nucleus [69] is not shown.

Fsy1 [5–7]. In addition to regulating the uptake of alternative sugars, the main glucose repression pathway prevents futile cycling in carbohydrate metabolism by shutting down *de novo* synthesis of glucose by the gluconeogenic pathway.

Regulation of the hormone-like effects of glucose and sucrose

Another principal carbon signaling pathway, the Ras/cAMP/PKA pathway, controls the expression of various genes involved in metabolism, proliferation and stress resistance in response to glucose (Figure 2 and reviewed in Refs [4,8,9]). Because full activation of this pathway requires extracellular glucose or sucrose [10], other sugars such as fructose, maltose, maltotriose and galactose cannot trigger a strong cAMP/PKA response [10,11] (Figure 2).

Among the targets regulated by the Ras/cAMP/PKA pathway are genes encoding heat-shock proteins, such as *HSP12* and *HSP104*, which are rapidly repressed on activation of this pathway [12,13] (Figure 3). These proteins have important roles in various processes that help yeast cells to cope with a broad array of stresses, including heat and ethanol stress [14–16]. Furthermore, high PKA activity also causes repression of the *TPS1* and *TPS2* genes encoding trehalose synthase [17]. Trehalose has a prominent role in cellular stress resistance because it protects membranes from desiccation and prevents protein denaturation [18].

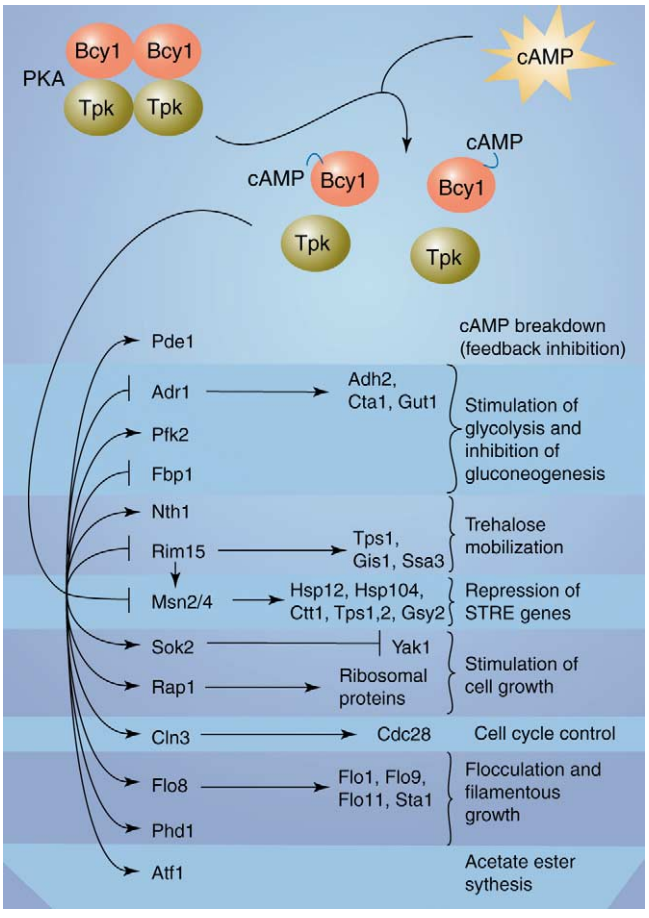

Figure 3. Targets of the Ras/cAMP/PKA nutrient signaling pathway. Targets include key proteins involved in the control of cell growth, glucose metabolism, stress resistance (including trehalose metabolism), flocculation and filamentous growth, and volatile ester synthesis, as well as feedback mechanisms. For example, one target of this pathway is the phosphodiesterase Pde1, which is responsible for the rapid breakdown of cAMP [4].

Glucose and sucrose signaling in industrial fermentations

In most commercial processes, the fermentation medium is a complex mixture of different fermentable sugars. The main sugars in grape must are glucose and fructose; by contrast, beer wort contains glucose, fructose, sucrose, maltose and maltotriose, and the fermentation medium for bioethanol production is usually a mixture of any of these sugars in variable concentrations, depending on the origin of the molasses [19,20].

In most applications, the initial concentration of glucose and/or sucrose in the growth medium is above the threshold concentrations (20–40 mM) for inducing the sugar signaling cascades [21,22]. Thus, both the main glucose repression pathway and the Ras/cAMP/PKA pathway are triggered at the start of the process. Activation of these pathways has three main consequences: repression of respiration, arrest of the consumption of other carbohydrates, and loss of cellular stress resistance. Although these effects presumably help *S. cerevisiae* to survive in its natural habitat, sugar signaling causes several problems in various yeast-based industrial processes.

The negative effects of catabolite repression on yeast performance

In an industrial context, one of the best-known adverse effects of the main glucose repression pathway on yeast performance is seen in the production of baker's yeast, during which the switch from respiration to fermentation induced by glucose or sucrose causes a marked drop in biomass yield. Similarly, in the production of biopharmaceuticals, repression of respiration decreases the yield of product [23,24]. Furthermore, the ethanol generated during fermentation is an additional stress factor for the yeast cells and can also negatively affect the stability of the pharmaceutical product. To avoid these effects, yeasts are grown mostly in aerated fed-batch reactors, in which the glucose and sucrose concentrations are maintained constantly below the threshold concentration for inducing the Crabtree effect [23,25].

By contrast, the production of alcoholic beverages and bioethanol requires fermentation. Apart from the first few hours, these fermentations are carried out anaerobically so that, even in the absence of the Crabtree effect, the yeast cells cannot respire. In this set-up, the negative effect of the main glucose repression pathway lies in the repressed uptake and metabolism of other sugars, such as galactose, maltose and maltotriose. The resulting reduced consumption of alternative sugars negatively affects the fermentation rate [26]. Remarkably, catabolite repression is not always relieved immediately once glucose and sucrose are depleted; instead, an initial preincubation in glucose leads to the slower metabolism of other sugars over several hours [21].

Empirical data obtained in various breweries show that extended propagation and cultivation in sucrose often leads to a permanent loss of maltose-fermenting capacity. Similarly, it has been recently shown that several genes encoding maltose transporters and maltase remain repressed when yeast cells are inoculated into maltose-containing medium after a long-term cultivation in glucose-rich medium [27]. This continued repression suggests that sustained favorable growth conditions, such as the availability of glucose, might cause a long-term decrease in the capacity of yeast to metabolize alternative carbon sources.

The negative effects of Ras/cAMP/PKA activation on yeast performance

As mentioned above, in addition to catabolite repression, glucose and sucrose also trigger activation of the Ras/cAMP/PKA pathway, which in turn leads to a decrease in the stress resistance of yeast cells. During industrial processes, yeast cells encounter various severe stress conditions, including shear stress; marked shifts in oxygen levels, temperature, osmolarity and pH; high ethanol and carbon dioxide concentrations; and a lack of nutrients [28,29]. Notably, Brosnan *et al.* [30] and Rossignol *et al.* [31] have reported that industrial yeast strains do not fully activate several stress responses during the first phases of industrial fermentations, which could be due to the presence of glucose and sucrose.

The inability of yeast cells to respond to unfavorable, stressful conditions leads to sluggish fermentations and

cell autolysis [32]. The latter process causes severe off flavors in alcoholic beverages, including a highly unpleasant goat-like aroma caused by the release of fatty acids, and a decrease in fruity aromas owing to the hydrolysis of volatile esters by esterases leaking out of damaged cells [33,34]. Furthermore, sustained high concentrations of glucose cause a decrease in the replicative lifespan of yeast, whereas the use of maltose-rich media generally leads to an increase in cell longevity [35,36]. Other reported effects of high glucose include an increase in the instability of short chromosomes [37] and a strong increase in the production of aroma-active esters through activation of the Ras/cAMP/PKA pathway [38,39]. Although moderate levels of these volatile esters are essential for the desired fruity flavors of beer and wine, high glucose concentrations lead to an undesirable overproduction of these compounds [39].

In addition to the Ras/cAMP/PKA, other closely related nutrient signaling pathways, such as the 'target of rapamycin' pathway and the 'fermentable growth medium-induced' pathway can influence the fermentation performance of yeast. In contrast to the short-term response of the Ras/cAMP/PKA pathway, which essentially responds to changes in glucose and sucrose levels, these pathways are thought to integrate the overall nutritional and/or energy status of cells [4,40,41]. This implies that, even in the presence of glucose and sucrose, some cellular stress responses can be activated when other essential nutrients (e.g. nitrogen sources) are depleted, as can occur in the last phase of wine fermentations [42]. Given the limited data, however, it is premature to estimate the importance of these signaling cascades in industrial processes.

Yeast carbon signaling mutants with improved industrial performance

Considering the various adverse effects of carbohydrate signaling on the performance of industrial yeasts, the use of strains with altered responses to glucose and sucrose should lead to a significant improvement in productivity. In addition, mutations in the regulatory systems might provoke 'balanced' changes in the expression of several genes involved in fermentation performance [43,44].

Indirect phenotypic evidence suggests that many of the currently used industrial strains have already acquired altered sugar signaling properties. Some brewer's strains, for example, do not break down trehalose in the presence of glucose [45]. Furthermore, many brewer's strains show constitutive uptake of maltose, independent of the presence of glucose [21]. Interestingly, in most cases glucose-mediated repression of the uptake of other less-preferred sugars, such as galactose, is still present. This indicates that if the alternative sugar-usage patterns are caused by alterations in the glucose-sensing pathways, these alterations are most probably present in a downstream part of the pathway [21]. Taken together, many industrial strains have acquired alternative glucose signaling phenotypes, enabling them to complete their tasks better in industrial processes.

Most of these yeasts are not the result of a specific, intentional selection procedure, but have presumably outcompeted the ancestral parent strain over long periods of time under stressful industrial conditions [46]. Although this natural selection can lead to vast improvements in yeast performance, especially when yeasts are re-used for multiple fermentations, as they are in beer production, further improvements can be achieved by a more direct approach; for example, the use of mutagenesis by ultraviolet irradiation or chemical mutagens such as ethyl methane sulfonate, followed by a screening or selection procedure. Alternatively, superior yeast strains can be developed by genetic engineering. Both methods have their pros and cons, which have been extensively discussed elsewhere [47,48]. Mutagenesis followed by selection does not require any knowledge of target genes or metabolic pathways and is relatively easy to perform. Furthermore, strains acquired through this process are not considered as 'genetically modified', which makes them virtually ready to use; however, a good selection procedure is required to facilitate rapid isolation of the few useful mutants from billions of 'uninteresting' cells. This selection requirement severely limits the use of this method for the isolation of yeasts with specific fermentation characteristics.

Many of the stress responses are not specific for particular stress conditions [13,16]; thus, a potential strategy is the selection of mutants that are more resistant to lethal stresses, such as heat or ethanol stress, in the presence of glucose or sucrose. After this first selection, the isolated mutants can be screened for improved fermentation properties under industrial stress conditions. In our experience, approaches in which the actual stressful fermentation conditions are mimicked as closely as possible greatly increase the chance of success [49]. Ideally, all screens should be therefore done in pilot-scale fermenters under growth conditions and medium similar to those used in the industrial-scale process. Even so, the mutant that performs best in pilot-scale trials is often not the best strain for full-scale production. Thus, it is vital to select several mutants for production-scale trials.

Recent progress in our understanding of the yeast carbohydrate signaling pathways makes it possible to improve strains by genetic engineering, leading to the introduction of such traits as increased trehalose levels and constitutive expression of targeted carbohydrate transporters [48,50,51]. Brewers and wine producers are reluctant to use genetically modified yeasts, however, mainly because of the complex legislation and the negative public perception. Furthermore, drastic changes in the vital carbon signaling pathways frequently affect other commercially important properties.

The mutations found in superior strains selected after random mutagenesis are often more subtle and can lead to more balanced performances [49,52]. Moreover, techniques similar to genome shuffling in bacteria [53] provide an efficient way with which to combine different subtle improvements. Thus, although genetic engineering and self-cloning are certainly the most promising methods for the future, random mutagenesis and selection remain the most realistic method with which to obtain improved yeast strains for short-term introduction into industrial food production.

These arguments do not apply to the production of heterologous proteins and biopharmaceuticals, in which the use of genetically modified organisms is less controversial. Moreover, the growth medium often contains only a single carbon source in these processes, and thus the metabolic and regulatory complications associated with complex sugar mixtures are avoided. A good example, developed and patented by Boles *et al.* [54], is a strain that has been genetically modified to express a chimeric hexose transporter. This strain is relieved of the Crabtree effect, and thus there is less need for sophisticated cultivation techniques to reach optimal production rates. Another interesting strain has been developed by Ostergaard *et al.* [43], who have used genetic engineering of the *GAL* regulatory system to increase galactose metabolism.

Selecting interesting carbon signaling mutants: a practical example

Relatively few carbon signaling mutants of industrial yeast strains have been described or investigated, in part, owing to the polyploid or aneuploid nature of these strains, which makes their genetic analysis much more difficult as compared with haploid laboratory strains. Perhaps the best examples of carbon signaling mutants are the so-called 'fermentation-induced loss of stress resistance' (*fil*) mutants of industrial baker's yeast, which show improved resistance to freezing and thawing in the presence of glucose and sucrose.

Freeze–thaw resistance is required for the increasingly popular use of frozen dough, which permits the separation of dough production and baking. Before freezing, yeast is mixed with the dough. Because of the high glucose and/or sucrose concentrations in the dough, however, yeast cells rapidly activate the Ras/cAMP/PKA pathway and lose their stress resistance, resulting in considerable loss of vitality during freezing and thawing [49,55]. By irradiating baker's yeast with ultraviolet, preparing dough with the mutants and subsequently subjecting the dough to numerous freeze–thaw cycles, it has been possible to isolate several mutants with improved freeze tolerance, although only a few strains retain all of the other desirable characteristics of industrial baker's yeast and perform superiorly in full-scale processes of dough freezing and thawing [49,56]. The exact mutations in these industrial strains are not known.

fil mutants have been also isolated from laboratory yeast strains, and two of these mutations have been identified. One strain contains a mutation in the *GPR1* gene, which encodes a G-protein-coupled receptor involved in glucose and sucrose sensing [57] (Figure 2). The other strain contains a partially inactivating mutation in the *CYR1* gene encoding adenylate cyclase, which is also an essential component of the Ras/cAMP/PKA pathway [44,52]. These mutations in Ras/cAMP/PKA signaling prevent the strains from losing their stress resistance in glucose and sucrose media. Remarkably, some of the laboratory *fil* strains were selected for heat-resistance in glucose medium, but the superior mutants also show improved resistance to various other stresses [44,52].

A healthy diet for optimal performance?

The selection or creation of superior yeast strains is not the only way to improve the productivity of industrial yeasts. Relatively simple changes in yeast handling can also lead to significant improvements. Considering the many adverse effects of glucose and sucrose on yeast performance, it is advisable to avoid using high concentrations of these sugars. In many applications, the composition of the fermentation medium cannot be changed without affecting product quality and cost, but sometimes it is possible to use alternative carbon sources. For example, when high-carbon syrups are added to the medium, as is often done in high-gravity beer fermentation or the production of bioethanol or biopharmaceuticals, it might be better to use maltose-rich syrups instead of sucrose-containing syrups.

Furthermore, when yeasts are propagated for industrial use, it is beneficial to combine glucose or sucrose with alternative sugars, such as maltose syrups, or with non-fermentable carbon sources, such as mannitol or sorbitol [58]. The preferred sugars will be used first, facilitating maximal biomass production rates, whereas the maltose or mannitol will be metabolized just before the production phase or storage of the yeast cells. Avoiding contact with glucose and sucrose just before the fermentation process might lead to increased levels of stored carbohydrates, better stress resistance and a faster fermentation rate. In the specific case of bioethanol production, it has proved advantageous to remove sugar monomers continuously from the saccharification vessel to maximize the breakdown and use of more complex sugars [46].

Conclusion

In the constant strive for better and more efficient yeast-based industrial processes, much attention has been drawn to various high-tech solutions ranging from optimization of bioreactors to the refinement of genetic technologies. The recently unraveled negative effects of glucose and sucrose on the industrial performance of yeast are often overlooked, and these two sugars remain among the most commonly used carbon sources in yeast media.

In general, the presence of these sugars at moderate concentrations is inevitable and will not cause significant problems. In some applications, however, high concentrations of glucose or sucrose at a particular stage of the industrial process cause a significant and long-term decrease in fermentation performance. Thus, the use of sugar signaling mutants or the reduction of glucose and sucrose might be advantageous in various bioindustries, ranging from the production of alcoholic beverages and bread to the fabrication of biopharmaceuticals. Indeed, although yeast cells seem to prefer glucose and sucrose over other carbohydrates, offering them too much of this 'fast-food' negatively affects their general fitness, again demonstrating the striking analogy between yeasts and higher eukaryotes.

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