Physiological functions of hexose transport and hexose phosphorylation in Saccharomyces cerevisiae.
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Citation for published version (APA):
Chapter 8

Co-consumption of sugars or ethanol and glucose in a *Saccharomyces cerevisiae* strain deleted in the *HXK2* gene

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8.1 Summary

In Chapter 6 it was shown that deletion of the \textit{HXX2} gene in \textit{Saccharomyces cerevisiae} yields a strain that hardly produces ethanol and grows almost exclusively oxidatively in the presence of abundant glucose. This chapter reports on physiological studies on the \textit{hxx2} mutant on mixtures of glucose/sucrose, glucose/galactose, glucose/maltose and glucose/ethanol in aerobic batch cultures. The \textit{hxx2} mutant co-consumed galactose and sucrose together with glucose. In addition, co-consumption of glucose and ethanol was observed during the early exponential growth phase. In \textit{S. cerevisiae}, co-consumption of ethanol and glucose (in the presence of abundant glucose) has never been reported before. The specific respiration rate of the \textit{hxx2} mutant growing on the glucose/ethanol mixture was 900 $\mu$mol-min$^{-1}$-(g protein)$^{-1}$, which is 4 to 5 times higher than that of the \textit{hxx2} mutant growing oxidatively on glucose, 3 times higher than its parent growing on ethanol (when respiration is fully derepressed) and is and almost 10 times higher than its parent growing on glucose (when respiration is repressed). This indicates that the \textit{hxx2} mutant has a strongly enhanced oxidative capacity when grown on a mixture of glucose and ethanol.
8.2 Introduction

*Saccharomyces cerevisiae* or bakers’ yeast ferments under aerobic conditions and is therefore a Crabtree-positive yeast. Aerobic fermentation is a consequence of a phenomenon called glucose repression. In the presence of high concentrations of glucose the expression of genes involved in the tricarboxylic acid (TCA) cycle, oxidative phosphorylation, glyoxylate cycle, gluconeogenesis and metabolism of sugars other than glucose is repressed (for recent reviews: (22, 23, 58)). Additionally, the expression of genes involved in alcoholic fermentation is induced. This regulatory mechanism in *S. cerevisiae* results in the preferential consumption of glucose over other carbon sources. Thus, when *S. cerevisiae* grows on a mixture of glucose and another carbon source such as sucrose, maltose, galactose or ethanol, growth is diauxic, i.e. glucose is metabolized first, whereas the other carbon sources are not metabolized until glucose is exhausted. Only after all sugars have been depleted, the ethanol produced during the fermentation of the sugars is consumed.

*S. cerevisiae* contains three distinct hexose-phosphorylating enzymes, hexokinase I (encoded by *HXK1*), hexokinase II (encoded by *HXK2*) and glucokinase (encoded by *GLK1*). Hexokinase I and II phosphorylate fructose as well as mannose and glucose, whereas glucokinase is only able to phosphorylate glucose and mannose. Each isozyme has a different affinity for glucose and ATP and is subject to a different mechanism of transcriptional regulation, depending, among others, on the concentration and the kind of carbon source available (72). Unlike in most other species, the activity of the hexokinases in *S. cerevisiae* is not inhibited by their metabolic product, glucose-6-phosphate, but appears to be regulated by trehalose-6-phosphate which is assumed to regulate the flux through the upper part of glycolysis (207, 212).

In many previous studies, it has been shown that hexokinase II is involved in glucose repression (for reviews see: (22, 23, 58)). Nevertheless, the exact role is of hexokinase II in glucose repression is still a matter of debate. On the one hand it is thought that deletion of *HXK2* causes relief of glucose repression as a consequence of the reduced hexose phosphorylating capacity (116, 182); on the other hand a direct regulatory role has been ascribed to hexokinase II (43, 44). In favour of the last proposal, hexokinase II is a phosphoprotein *in vivo* (234) which can exist in either a dimeric or monomeric form. The oligomerisation state of hexokinase II shifts to the monomeric state by phosphorylation which is initiated at low glucose (10). The phosphorylated form of hexokinase II was recently shown to enter the nucleus (170), the nuclear protein participates in a DNA-protein complex which regulates repression of at least the *SUC2* gene by glucose (73). The phosphorylation of hexokinase II at low glucose (10) contradicts the results that show that phosphorylation of this enzyme is necessary to enter the nucleus and initiate glucose repression (73, 97). At this moment, the exact mechanism by which hexokinase II enters the nucleus and causes repression of at least *SUC2*, is unclear.

Early characterisation of an *S. cerevisiae* strain deleted in the *HXK2* gene showed that the absence of hexokinase II relieved glucose repression of the indicator gene *SUC2*, encoding invertase (42, 116, 128). Further, the genes encoding the maltose transporter and maltase (257) and the genes encoding the high-affinity glucose transporters Hxt2p and Hxt7p were shown to be derepressed (see Chapter 5 and (245)). DNA arrays performed on an *hxk2*
deletion strain grown with excess glucose showed clearly that the expression of many genes involved in glucose repressible routes such as the TCA cycle, glyoxylate cycle, oxidative phosphorylation and the consumption of sugars other than glucose were up-regulated whereas the expression of those gene products involved in glycolysis were down-regulated (unpublished results). Accordingly, the *hxk2* deletion strain displayed an increased flux through the TCA cycle and oxidative phosphorylation in the presence of excess glucose. The flux through glycolysis and the fluxes to ethanol and glycerol were decreased resulting in almost exclusively oxidative growth and diminished ethanol production (see Chapter 6). The growth yield of the *hxk2* deletion strain during aerobic growth on glucose is much higher than that of its parent strain since fermentation of glucose to ethanol yields only two ATP per glucose consumed, whereas complete oxidation of glucose to carbon dioxide and water yields roughly 20 ATP.

The derepression of the genes encoding enzymes involved in maltose metabolism (*MAL* genes) and sucrose metabolism (*SUC2*) might suggest that the *hxk2* deletion strain is also capable of consuming sucrose and maltose in the presence of abundant glucose. Therefore, in continuation of the physiological characterisation of the *hxk2* deletion strain in Chapters 6 and 7, the performance of this strain was studied on mixed carbon sources, i.e. mixtures of glucose with either sucrose, galactose, maltose or ethanol both on plates and in batch cultures.

### 8.3 Materials and Methods

**Strains** – The wild-type *Saccharomyces cerevisiae* strain X2180-1A (*MATα SUC2 mal mel*) was obtained from the Yeast Genetic Stock Center in Berkeley, California. The *S. cerevisiae* prototrophic wild-type strain CEN.PK113-7D (*MATα MAL2-8c SUC2*) obtained from P. Kötter (Johann Wolfgang Goethe Universität, Frankfurt, Germany) was used to create a strain KY116 in which the *HXK2* gene was deleted as follows: using primers AK53 (GTT G TAGGAATATAATTCTCCACACATAATAAGTACGCTAATTCGTACGCTGCAGGTC A A) and AK54 (AAAAGGGCACCrTCTTGTTGTTCAAACTTAATTTACAAATTAAGT A TCGATGAATTCGAGCTCG), the kanMX cassette of plasmid pFA6a-kanMX4 (235) was amplified using the Expand PCR kit as recommended by the manufacturer (Roche). The resulting PCR product was transformed into competent CEN.PK113-7D as described (61). After 2 hours of cultivation in YPD medium (1% w/v yeast extract, 2% w/v peptone, 2% w/v glucose), the transformed cells were plated on solid YPD medium (2% w/v agar) containing G418 (200 μg·ml⁻¹) and incubated at 30°C. G418-resistant isolates were tested for proper integration of the kanMX cassette at the *HXK2* locus by analytical PCR using the TaqPlus Long PCR kit with the primers AK60 (GACGAAATACGCGATCGCTGT) and AK61 (GCCGAACATTTCAAAGTCAACC) as recommended by the manufacturer (Stratagene).

**Plate assays** – Solid medium contained 2% (w/v) of each carbon source and 1 mM 5-thio-D-glucose in YNB medium which consisted of 0.17% (w/v) yeast nitrogen base w/o amino acids (Difco) with 0.5% (NH₄)₂SO₄. The strain X2180 was supplemented with casamino acids.

**Co-consumption batch experiments** – The yeast strains were cultivated in batch fermentors at 30°C. The cells were pregrown in 20 ml of 1% (w/v) glucose-YNB medium in
100 mM potassium phthalate at pH 5.0. The next day, fermentors were inoculated containing 1 litre of fresh medium with 1% (w/v) glucose plus 0.4% (w/v) sucrose, 0.4% (w/v) galactose, 0.4% (w/v) maltose, or 100 mM ethanol. The \textit{hxk2} mutant and the isogenic parent strain were cultivated on each of these carbon mixtures. To exclude the possibility that the onset of fermentation is caused by oxygen limitation, the fermentors were aerated at 1 vessel volume per minute and stirred at 1000 rpm. The cells were grown overnight and samples were taken the next day during and beyond the exponential phase. Growth was monitored by measuring the optical density at 600 nm.

Extracellular metabolites were measured by spinning down 1 ml of culture and injecting the supernatant into 100 µl of 35% (v/v) PCA at 0°C. After 15 minutes, part of the PCA was precipitated by adding 55 µl of 7 M KOH. After centrifugation the supernatant was filtered and analysed for glucose, ethanol, glycerol, acetate and pyruvate by HPLC (see Materials and Methods Chapter 6).

Oxygen consumption and carbon dioxide production were determined by passing the gas from the fermentor through an oxygen analyser (Taylor Servomex Type OA 272) and a carbon dioxide analyser (Servomex IR Gas Analyser PA 404). The protein content of the culture was measured according to Lowry \textit{et al.} (115) using bovine serum albumin (fatty-acid free) as a standard and measured on a COBAS-FARA automatic analyser (Roche).

The RQ equals the quotient of CO\textsubscript{2} production to O\textsubscript{2} consumption. When \textit{S. cerevisiae} completely oxidises a fermentable carbon source, i.e. glucose to CO\textsubscript{2} and water via the TCA cycle, the RQ equals 1, because for each molecule of CO\textsubscript{2} produced one molecule of O\textsubscript{2} is consumed. When a fermentable carbon source is partially fermented and partly oxidised the RQ will reach values (much) larger than 1. When \textit{S. cerevisiae} grows on ethanol the RQ has the specific value of 0.67 (114).

### 8.4 Results

**Glucose repression through \textit{HXK2}; revisited** – The glucose analogue 5-thioglucone is known to induce glucose repression in \textit{S. cerevisiae}, yet cannot be metabolised after being taken up by the cell (39). Consequently, a wild-type \textit{S. cerevisiae} strain will not be able to grow on medium containing both 5-thioglucone and another carbon source such as sucrose, galactose, maltose or ethanol since the required pathways and enzymes are repressed. In the presence of 5-thioglucone, metabolism in the isogenic wild-type strain is glucose repressed and therefore it cannot consume either sucrose or ethanol/glycerol in the medium, whereas glucose can still be consumed (Fig. 8.1). Galactose consumption does not seem to be strongly glucose repressed in the parent strain since quite some growth appeared on galactose in the presence of 5-thioglucone. This may be explained by the fact that expression of genes whose products are involved in galactose metabolism are not only regulated by glucose repression but can also be induced by the presence of galactose (140). Due to the constitutive expression of the \textit{MAL} genes in the CEN.PK113-7D strain, growth of the parent strain on maltose in the presence of 5-thioglucone was not inhibited (Fig. 8.1). Further, growth on maltose in the presence or absence of 5-thioglucone was reduced in the \textit{hxk2} deletion strain compared to its parent (Fig. 8.1). In the X2180-1A strain the \textit{MAL} genes seem glucose repressible and, accordingly, growth on maltose is impaired in the presence of 5-thioglucone whereas in the absence of 5-thioglucone growth in both the mutant strain and its parent is restored. These results show that this phenotype is strain dependent (Fig. 8.1).
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The hkk2 deletion strain is capable of growth in the presence of 5-thioglucose on each of the carbon sources tested (Fig. 8.1). Thus, the enzymes involved in the metabolism of sucrose, galactose, maltose, and ethanol/glycerol were expressed in the hkk2 deletion strain in the presence of 5-thioglucose. This is confirmed by the fact that invertase, galactose permease, maltase, and maltose permease are derepressed in the hkk2 deletion strain (42, 46, 128, 167, 168). Moreover, genes involved in ethanol/glycerol metabolism are expressed as well. Additionally, DNA arrays performed on the hkk2 deletion cells grown on glucose showed that ALD2 (aldehyde dehydrogenase) and ACSI (acetyl-coA synthetase), both involved in ethanol metabolism, are up-regulated in the mutant (data not shown). Surprisingly, ADH2 (alcohol dehydrogenase) whose product catalyses the conversion of alcohol to acetaldehyde, was down-regulated, suggesting that ethanol needs to be present for the up-regulation of ADH2. The same is true for the gluconeogenic enzyme fructose-1,6-bisphosphatase which is not expressed in the hkk2 deletion strain growing on glucose, but which must be expressed on ethanol/glycerol plus 5-thioglucose to form sugar phosphates from either glycerol or ethanol for further anabolism.

Deletion of HXK2 enhances oxidative metabolism; co-consumption of glucose and sucrose — The physiology of the hkk2 deletion strain co-consuming glucose with either sucrose, galactose, maltose or ethanol was studied in greater detail and more quantitatively in aerated batch fermentors. To obtain a better insight in the distribution of the carbon fluxes over the fermentative and the oxidative routes the respiratory quotient (RQ) was calculated during the entire batch growth.

The CO₂ production, O₂ consumption, and RQ of the parent strain grown in an aerated batch fermentor on a mixture of glucose and sucrose were measured as a function of the time after inoculation (Fig. 8.2A). Growth on glucose yielded an RQ of approximately 9, confirming respiro-fermentative growth. After 20.5 h glucose had been completely consumed and the shift to growth on sucrose was made as can be observed from the small decrease in
CO₂ production and O₂ consumption rates. Subsequently, the value of the RQ was about 6, indicating fermentative growth and thus repressed metabolism but less severely than during growth on glucose. Growth on sucrose was rather brief (1 to 2 h) since the biomass concentration was high and the original sucrose concentration added to the medium was relatively low. After approximately 22 h, the sucrose was exhausted and the shift to growth on ethanol set in. Accordingly, the CO₂ production and to a lesser extent the O₂ consumption rates collapsed. Within a few hours metabolic fluxes were re-established with an RQ of 0.6 which is characteristic for growth on ethanol. At approximately 40 h the RQ rose from 0.6 to 1 which is indicative for growth on acetate after the ethanol has been exhausted. After 42 h the RQ values scattered as growth had ended and the O₂ consumption was almost zero. The scatter of the RQ within the first 20 hours was caused by the low oxygen consumption rate due to glucose repression in combination with a low biomass concentration.

Figure 8.2 The gas-profiles of the wild-type strain and hxx2 deletion strain growing in batch on a mixture of glucose and sucrose. The respiratory quotient (x) (RQ = CO₂ / O₂), the CO₂ production rate (•) and the O₂ consumption rate (O) per litre culture (% gas-min⁻¹(litre culture)⁻¹) in the wild-type strain (A) and the hxx2 deletion strain (C). The specific CO₂ production (•) and O₂ consumption (O) (µmol-min⁻¹(g protein)⁻¹) of a part of the growth curve of the wild-type strain (B) and the hxx2 deletion strain (D).
Figure 8.3 The gas-profiles of the wild-type strain and hxxk2 deletion strain growing in batch on a mixture of glucose and ethanol. The respiratory quotient (R) (RQ = CO₂ / O₂), the CO₂ production rate (•) and the O₂ consumption rate (O) per litre culture (% gas·min⁻¹·litre culture⁻¹) in the wild-type strain (A) and the hxxk2 deletion strain (C). The specific CO₂ production (•) and O₂ consumption (O) (µmol·min⁻¹·(g protein)⁻¹) of a part of the growth curve of the wild-type strain (B) and the hxxk2 deletion strain (D).

The RQ profile, the CO₂ production rate and O₂ consumption rate in the hxxk2 deletion strain are very different from the wild-type (Fig. 8.2C). First, there is no discernible shift from glucose to sucrose, which suggests that the sucrose is simultaneously metabolised with the glucose. Fig. 8.4B indeed shows that sucrose is already immediately hydrolysed by derepressed invertase activity to glucose and fructose. The fructose formed was not metabolised until practically all glucose had been consumed. This is not caused by glucose repression but is a consequence of the lower affinity of the hexose transporters for fructose than for glucose (174). The parent strain, however, does not hydrolyse the sucrose before all glucose has been consumed (Fig. 8.4A).

Secondly, the growth of the hxxk2 deletion strain on ethanol (from approximately 30 to 37 h) takes only half the time of that of the parent strain (from 24 to 38 h) (compare Figs. 8.2A and 8.2C). This is caused by the much lower ethanol production during the predominantly oxidative growth on glucose and sucrose by the hxxk2 deletion strain, whereas the wild-type strain converts glucose mainly to ethanol. In the first 22 hours, the RQ of the hxxk2 deletion strain was close to 1 which is characteristic of fully respiratory growth (Fig. 8.2C). Then, fermentation set in at around 23 h and the RQ rose above 1 and gradually increased further to almost 3 before glucose and sucrose were exhausted at 28 h.

The specific respiration rates of the parent strain (Fig. 8.2B) and hxxk2 deletion strain (Fig. 8.2D) during exponential growth with glucose (and sucrose) as carbon source, show that
the specific CO₂ production rate is much higher in the parent than in the mutant, 900 μmol-min⁻¹·(g protein)⁻¹ and 600 μmol-min⁻¹·(g protein)⁻¹, respectively. The specific oxygen consumption is approximately 150 μmol-min⁻¹·(g protein)⁻¹ in the parent and maximally 450 μmol-min⁻¹·(g protein)⁻¹ in the hxlk2 deletion strain.

Figure 8.4 Growth on a mixture of glucose and sucrose. The extracellular sugar concentrations (mM) during growth of the wild-type strain (A) and the hxlk2 deletion strain (B) on a mixture of glucose (■) and sucrose (○) and fructose (△).

Co-consumption of glucose and galactose or maltose – Growth of both the parent and the hxlk2 deletion strain growing batch-wise on glucose plus galactose essentially showed the same RQ, CO₂ and O₂ profile as Figs. 8.2A and 8.2C (results not shown). In the hxlk2 deletion strain the co-consumption of glucose and galactose was obvious: the extracellular galactose concentration was already decreasing when glucose was still abundant (Fig. 8.5B). In the parent first the glucose was exhausted (at 18 h) after which the galactose was consumed (Fig. 8.5A). At 23 h the galactose was exhausted and the shift to growth on ethanol set in.

The parent strain did not have any difficulty growing on a mixture of glucose and maltose, first the glucose was consumed and then the maltose was taken up and hydrolysed to glucose (Fig. 8.6A). The hxlk2 deletion strain, however, grew poorly on a mixture of maltose and glucose (or maltose alone) with a μ_max of 0.12 h⁻¹ instead of 0.32 h⁻¹ on other fermentable carbon sources (data not shown). Nevertheless, maltose concentrations decreased, indicating uptake and intracellular hydrolysis of maltose (Fig. 8.6B). Simultaneously, the extracellular glucose levels increased slightly around 16 to 18 h, indicating that the maltose was hydrolysed faster than it was metabolised resulting in the export of intracellular glucose into the medium by the available hexose transporters. After all the maltose had been hydrolysed, the glucose was consumed next and growth was restored. The poor growth in the presence of maltose was not due to ATP depletion as a consequence of uncontrolled active transport of maltose over the membrane, since the intracellular ATP concentration was approximately 8 mM and the ATP/ADP ratio approximately 5.
**Figure 8.5** Growth on a mixture of glucose and galactose. The extracellular sugar concentrations (mM) during growth of the wild-type strain (A) and the hxxk deletion strain (B) on a mixture of glucose (■) and galactose (○).

**Figure 8.6** Growth on a mixture of glucose and maltose. The extracellular sugar concentrations (mM) during growth of the wild-type strain (A) and the hxxk deletion strain (B) on a mixture of glucose (■) and maltose (○).

**Co-consumption of glucose and ethanol** – On a mixture of glucose and ethanol the parent consumed the glucose first and converted the glucose mainly to ethanol; only after glucose exhaustion ethanol was consumed (Figs. 8.3A and 8.7A). Interestingly, in the early to mid exponential stages of growth, the ethanol concentration in the medium of the hxxk deletion strain decreased (Fig. 8.7B). This suggests consumption of ethanol, but might be an artefact caused by evaporation of ethanol in the vigorously aerated fermentor. However, the gas data revealed that the RQ value was lower than 1, which irrefutably shows that ethanol was actually consumed simultaneously with glucose. The RQ gradually increased and at 18 h the value became larger than 1 and net fermentation set in. Thus the hxxk deletion strain consumed ethanol in the presence of abundant glucose in early exponential growth, then switched to ethanol production during late exponential growth, then switched back to ethanol consumption after the glucose was exhausted (Figs. 8.3C and 8.7B). The parent strain
produced ethanol due to fermentation of glucose (Fig. 8.7A) which agrees with the high RQ values of 8 until glucose was exhausted (Fig. 8.3A). The specific oxygen consumption by the hxx2 deletion strain was extremely high and reached values of almost 900 μmol-min⁻¹-(g protein)⁻¹ compared to 200 for the parent (Fig. 8.3B). Even during fully oxidative growth of the parent on ethanol this value reached only 300 μmol-min⁻¹-(g protein)⁻¹ (Fig. 8.2B).

![Figure 8.7 Growth on a mixture of glucose and ethanol. The extracellular glucose (■) and ethanol (⊡) concentration (mM) during growth of the wild-type strain (A) and the hxx2 deletion strain (B).](image)

**8.5 Discussion**

This chapter reports on the physiological behaviour of *S. cerevisiae* strains deleted in the HXXK2 gene during growth on mixtures of carbon sources. The hxx2 deletion strain was investigated for co-consumption properties both on plates and, in more detail, in batch fermentors. The plate assays revealed that the hxx2 deletion strain could grow on sucrose and even on ethanol/glycerol in the presence of the glucose analogue 5-thioglucose whereas the parent could not (Fig. 8.1). Comparable, more quantitative results were found in the batch fermentors. In batch cultures, the hxx2 deletion strain co-consumed sucrose with glucose (Fig. 8.4B), galactose with glucose (Fig. 8.5B) and ethanol/glycerol together with glucose (Fig. 8.7B) of which the latter could be confirmed by the RQ value which was smaller than 1 (Fig. 8.3D).

It was observed consistently that the specific oxygen consumption in the hxx2 deletion mutant decreased in time (Figs. 8.2D and 8.3D). Recently, we found that the requirement for biotin in the hxx2 deletion strain is higher than in its parent (167, 168). The gradual decrease in specific respiration rate can be (partly) prevented by the addition of extra biotin to the standard growth medium.

In liquid medium and on plate, the hxx2 mutant consumed galactose in the presence of glucose and 5-thioglucose, respectively. The parent strain, however, grew in the presence of 5-thioglucose on plates as well, thus galactose metabolism was not fully repressed. In the batch fermentor, the parent strain could not consume glucose together with galactose (Figs. 8.1 and 8.5A). This discrepancy might be caused by the different concentration of galactose in the medium or the different molar ratio of glucose (or 5-thioglucose) to galactose. On plates a higher concentration of galactose (2%) was present than in the fermentors (0.4%). The higher galactose concentration in the solid medium may be sufficient to induce galactose metabolism and overrule the glucose repression in the parent strain whereas the lower initial
galactose concentration in the batch fermentor may not have been able to overrule glucose repression.

Remarkably, the X2180-1A strain was capable of growth on maltose (Fig. 8.1), which is in contrast to earlier observations (e.g. Chapter 4). The X2180-1A is characterised as mal minus, however it is not clear whether this characterisation is based on phenotypological tests or addresses to its genotype. The absence of maltose uptake and growth on maltose in liquid medium that was found for the diploid strain as described in Chapter 4, in contrast to the growth on plates of the haploid described in this chapter, might be related to differences in preceding growth conditions. We presume that the X2180 strains are able to grow on maltose, but induction of the MAL genes is dependent on the preceding growth conditions. However this hypothesis has to be tested further.

The hxxk2 deletion strain grew poorly on a mixture of glucose and maltose in liquid medium, first all maltose was taken up and hydrolysed before the glucose was consumed (Fig. 8.6B). The hydrolysed maltose was probably accumulated in the cytoplasm and subsequently transported out of the cell since the extracellular glucose concentration actually rose. On plates it could be observed as well that the hxxk2 deletion strain in the CEN.PK background grew worse on maltose in the presence of 5-thioglucose compared to its parent (Fig. 8.1), probably as a result of the constitutive expression of the MAL genes in this strain. The uncontrolled uptake of maltose by the hxxk2 deletion strain costs ATP, since maltose permease is an active transporter. This may suggest that the strain cannot grow well due to exhaustion of intracellular ATP. However, the ATP concentration was 8 mM and the ATP/ADP ratio was approximately 5, values which are even higher than the average on fermentable carbon sources. The hxxk2 mutant may show impaired growth as a result of osmotic damage.

A completely novel observation was the ability of an S. cerevisiae (with an HXX2 deletion) strain to simultaneously metabolise glucose and ethanol in the presence of excess glucose. Co-consumption of glucose and ethanol in S. cerevisiae has been reported but this could be only accomplished by using dual-substrate limited chemostat cultures (60). In such cultures, the glucose and ethanol consumption could be manipulated by changing the relative concentrations of glucose and ethanol in the medium feed.

The specific respiration rates measured in the batch fermentors varied strongly depending on the carbon sources available and the type of strain. The parent strain displayed respiration rates that were maximally 200 μmol-min\(^{-1}\)·(g protein\(^{-1}\)) in the presence of glucose, sucrose or galactose and 300 μmol-min\(^{-1}\)·(g protein\(^{-1}\)) during growth on ethanol (fully derepressed conditions; Figs. 8.2B and 8.3B, and Chapter 6). The hxxk2 deletion strain had an increased specific respiration rate on galactose and sucrose of approximately 450 μmol-min\(^{-1}\)·(g protein\(^{-1}\)), and on a mixture of glucose and ethanol attained 900 μmol-min\(^{-1}\)·(g protein\(^{-1}\)). Especially under these conditions where high biomass concentrations are present in combination with high specific growth rates, the specific respiration rates decline in time (Figs. 8.2D and 8.3D). This is most likely due to the earlier mentioned biotin requirement (167, 168).

During industrial processes in rich complex medium S. cerevisiae will first consume the most favoured carbon source and then switch to the next and so on. An S. cerevisiae strain, such as the hxxk2 mutant, that can co-consume different substrates would overall grow faster and is therefore appealing for use in processes such as the production of bakers’ yeast or heterologous proteins. Additionally, if most of the available sugars can be consumed in
industrial media, the environmental burden of squandering used media will clearly be relieved. The hxx2 mutant strain therefore appears to meet several of the properties desired for an *S. cerevisiae* strain to be used for commercial yeast biomass production or heterologous protein production.

**Acknowledgements**

We acknowledge Dr. J. Snoep for the fruitful discussions and Dr. H. de Winde for critically reading the manuscript. This work was supported by the Association for Biotechnological Research Schools in the Netherlands (ABON). A.L.K. was supported by an EU grant, no. BIO4-CT98-0562, DG12-SSM1.