Differential Gene Expression Profiles and Real-Time Measurements of Growth Parameters in *Saccharomyces cerevisiae* Grown in Microliter-Scale Bioreactors Equipped with Internal Stirring

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Combining real-time growth kinetics measurements with global gene expression analysis of microbial cultures is of significant value for high-throughput biological research. We have performed differential gene expression analysis in the eukaryotic model *Saccharomyces cerevisiae* grown in galactose and glucose media in 150 μL bioreactors equipped with sensors for in situ and real-time measurements of optical density (OD), pH, and dissolved oxygen (DO). The microbioreactors were fabricated from poly(dimethylsiloxane) (PDMS) and poly(methyl methacrylate) (PMMA) and equipped with internal magnetic ministirrers and evaporation compensation by water replacement. In galactose-grown cells, the core genes of the GAL operon GAL2, GAL1, GAL7, and GAL10 were upregulated at least 100-fold relative to glucose-grown cells. These differential gene expression levels were similar to those observed in large-scale culture vessels. The increasing rate at which complete genomic sequences of microorganisms are becoming available offers an unprecedented opportunity for comparative investigations of these organisms. Our results from *S. cerevisiae* cultures grown in instrumented microbioreactors show that it is possible to integrate high-throughput studies of growth physiology with global gene expression analysis of microorganisms.

Introduction

To effectively leverage the rapid advances in biochemical, metabolic, and in vitro engineering, there is a need for high-throughput biochemical platforms enabling real-time monitoring and analysis of microbial environmental and physiological parameters. Such platforms linked to global expression technologies (gene, proteins, metabolites) would provide whole-organism information invaluable for characterizing and designing biological systems.

Global genomic expression assays using DNA microarrays permit the exploration of the cells transcriptional state under a wide range of physiological parameters. Microarray analysis has been widely applied in basic biological research as well as in specialized fields, such as drug screening, environmental testing, and clinical diagnosis (Debouck et al., 1999; Bodrossy et al., 2004). The development of powerful global expression technologies has not yet been matched by biochemical platforms that support multiple parallel experiments with real-time assessment of microbial growth kinetics.

Moreover, biological analytical techniques for microbial cell growth have remained essentially unchanged for several decades. Conventional laboratory-scale cell culturing devices include small-scale (0.5−10 L) stirred tank bioreactors, shake flasks, test tubes, and microtiter plates. These analytical platforms yield limited physiological and metabolic data. An array of microliter-scale bioreactors, made with microfabrication techniques and scalable production methods such as soft lithography and injection molding, has the potential to address this problem by allowing multiple parallel microbial cultivations for high-throughput analysis. Significant advantages could arise from combining parallel instrumented microbioreactors with microarray technologies to yield high-throughput gene expression analysis.

Efforts have been made to demonstrate that growth physiology of bacteria and yeast in microliter-scale culturing devices is comparable to that in bench-scale growth vessels. Kensy et al. (2005) have optimized growth conditions for the yeast species *Hansenula polymorpha* in 48-well microtiter plates with culture volumes of 300−600 μL. Microtiter plates have been used in *S. cerevisiae* high-throughput systems to perform large-scale screen of the gene/protein functions of open reading frames from the yeast chromosome III (Rieger et al., 1997). Holz et al. (2002) utilized a 96-well microtiter plate high-throughput system to optimize protein production. Diaz-Camino et al. (2003) perfomed β-galactosidase assays in microtiter plates in a 2-hybrid screen to identify protein−protein interactions.

Nevertheless, small-scale, parallel culture systems face systematic problems that affect the type of data that can be obtained as well as the reproducibility of the experiments. For example, in the 96-well format the mixing of cultures requires shaking the entire plate, which puts engineering constraints on the type of instrumentation for fluidic connections, ports, and optical sensors to measure fermentation parameters in situ. Furthermore, microbial cultivations with extended incubations in microtiter plates can result in significant medium evaporation

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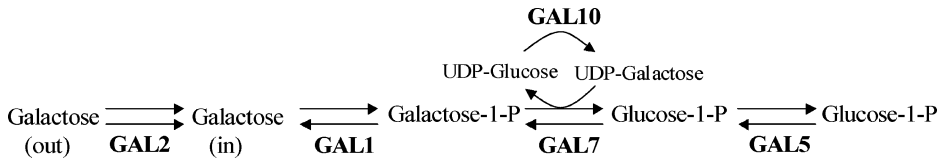

Figure 1. Pathway of galactose utilization. *Sacharomyces cerevisiae* metabolizes galactose in a series of steps that start with the transport of galactose into the cell via a permease (GAL2), followed by the transformation of galactose to glucose-6-P via a galactokinase (GAL1), a uridyl transferase (GAL7), an epimerase (GAL10), and a phosphoglucomutase (GAL5).

(Han et al., 2005) with large well-to-well volume variations and thus low reproducibility.

Microbioreactor technology with integrated sensors is a promising solution for rapid, high-throughput, and cost-effective screening. Kumar et al. (2004) reviewed recent progress in the area of minibioreactors as well as small-scale conventional bioreactors. One fundamental requirement for microliter-scale bioreactors is the ability to obtain optical density (OD), pH, and dissolved oxygen (DO) data in real time, thereby avoiding the need for sampling.

Recent developments in electrochemical and optical sensor technologies have made these measurements possible in microbioreactors. Van der Weide et al. (2003) correlated capacitance and resistance to the biomass in microbial cultures in wells with a liquid content of up to 30 μL. The microbioreactor of Maharbiz et al. (2003) is a hybrid of plastic microplate reactors combined with silicon technology; in the reactor, oxygen is generated and delivered by hydrolysis of water. Most recently, Maharbiz et al. (2004) integrated 250 μL microbioreactor arrays with OD and pH measurements on a commercial printed circuit board. Zanzotto et al. (2004) described a 50 μL membrane-aerated microbioreactor, fabricated out of poly(dimethylsiloxane) (PDMS) and glass, and demonstrated *E. coli* growth kinetics comparable with those of lab-scale culturing vessels. In this same platform we (Boccazzi et al., 2005) analyzed global gene expression analysis of *E. coli*. Zhang et al. (2006) reported growth kinetics of *E. coli* grown in a 150 μL bioreactor made of poly(methyl methacrylate) (PMMA) and PDMS with integrated optics for in situ and real-time measurements of OD, pH, and DO. The reactor was connected to a water reservoir in order to compensate for evaporation and contained an internal, miniaturized stirring bar for mixing the culture during fermentation.

Here we report the analysis of global gene expression profiles in *Saccharomyces cerevisiae* grown in glucose and galactose media in stirred microbioreactors (Zhang et al., 2006). *S. cerevisiae* is a model organism that has contributed greatly to our understanding of other more complex and less manipulable organisms (Lashkari et al., 1997); moreover, the galactose utilization pathway is one of the best studied in the budding yeast (Figure 1). The wealth of information on this pathway, combined with its relative simplicity, has made it a useful system to evaluate the reproducibility and sensitivity of novel techniques and tools (Ideker et al., 2001; Hood, 2003).

It is well estabilished that *S. cerevisiae* grown in galactose medium upregulates the GAL operon (Ideker et al., 2001; Hittinger et al., 2004), which comprises the genes responsible for the catabolism of galactose to glucose-6-P (Figure 1). The core genes that allow *S. cerevisiae* to utilize galactose as carbon source include GAL2, which encodes a permease for galactose transport into the cell, and GAL1, GAL7, GAL10, and GAL5, the structural genes for galactokinase, uridylyltransferase, epimerase, and phosphoglucomutase, respectively. The regulatory mechanism of the GAL operon is not fully understood in detail, but it is known that the three of the main regulators are encoded by GAL3, GAL80, and GAL4 (Johnston, 1987; Lohr et al., 1995; Ideker et al., 2001) and that other genes such PGM2 and GAL6 play an important role in galactose transport and utilization (Bro et al., 2005).

The experiments reported here were performed to demonstrate the technical feasibility and reproducibility of determining in real time the growth kinetics of *S. cerevisiae* in well-mixed 150 μL microbioreactors simultaneously at the physiological and molecular levels. The results demonstrate that an integrated microbioreactor platform for gene expression analysis for biological research and bioprocesses development can be utilized to obtain quantitative and reproducible results.

Materials and Methods

Organism and Growth Conditions. *Saccharomyces cerevisiae* ATCC 4126 was obtained from the American Type Culture Collection (ATCC, Manassas, VA) and used in all experiments. Cultures were grown at 30 °C in YPD or YPGal. The composition of YPD is 10 g/L yeast extract (Difco, BD Diagnostic Systems, Franklin Lakes, NJ), 5 g/L peptone (Difco), 10 g/L glucose (Sigma-Aldrich, St. Louis, MO), and 50 mg/L streptomycin (Sigma-Aldrich). YPGal is identical to YPD except that 10 g/L galactose (Sigma-Aldrich) was substituted for glucose. Reproducible inocula were obtained using a standardized inoculum procedure in which the strain was first adapted to YPD or YPGal as follows: 5 mL of each medium was inoculated with a single colony from an overnight YPD agar plate (containing streptomycin at 50 μg/L). The cultures were incubated at 30 °C on a roller drum at 60 rpm. Optical density (OD) measurements of diluted cultures were made at 600 nm with a Spectronic 20 Genesys spectrophotometer (Spectronic Instruments Inc., Rochster, NY). At an OD_{600} of about 1.0, 1.6 mL was used to inoculate 30 mL of fresh medium in 500 mL baffled shake flasks and incubated at 30 °C on a horizontal rotary shaker at 150−200 rpm until the optical density reached about 1.0. At this point the culture was diluted in fresh YPD or YPGal to reach an OD_{600} of about 0.05 and used to inoculate microbioreactors.

Microbioreactor Growth Cultures. Microbioreactors, utilized for all cultivations, consisted of two layers of poly(dimethylsiloxane) PDMS sandwiched between two layers of poly(methyl methacrylate) (PMMA) (Figure 2). The bottom PMMA layer included the microbioreactor chamber (diameter 10 mm, depth 2 mm) and three connecting channels (depth 500 μm, width 500 μm) that were used for inoculation and compensation for water evaporation and as a liquid outlet. A thin layer (100 μm) of spin-coated PDMS covered the chamber and served as the aeration membrane. The working volume of the microbioreactor was 150 μL. To facilitate device assembly, hermetic sealing, and connection of microfluidic channels, this PDMS layer was held by a 5 mm-thick PDMS gasket layer.

Two recesses at the bottom of the bioreactor chamber accommodated fluorescence lifetime sensors for dissolved oxygen (DO) and pH (DO sensor foil PSt3, and pH sensor solution HP2A, PreSens-Precision Sensing GmbH, Regensburg,

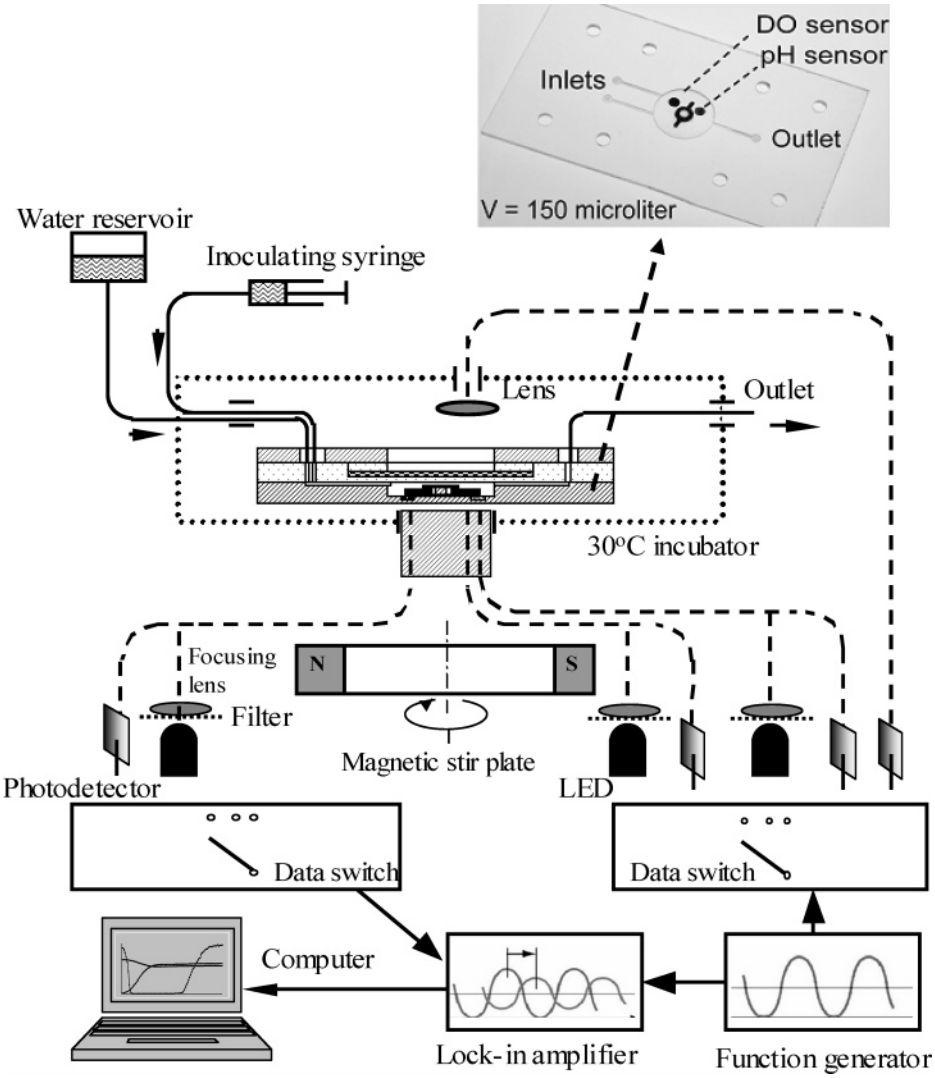

Figure 2. Schematic of longitudinal section and assembly of stirred microbioreactor made of PMMA and PDMS with fluorophore sensors for pH and dissolved oxygen (DO). Cell cultures in the microbioreactor (see picture insert) are stirred with a magnetic stir bar, which rotates around a vertical post in the center of the reactor.

Germany). A ring-shaped magnetic stir bar with 6 mm arm length and 0.5 mm thick (custom-made by Engineered Concepts, Vestavia Hills, AL) rotated around a vertical post (1.35 mm in diameter) machined out of the bulk PMMA in the center of the reactor chamber. A piece of PMMA, 250 μm thick and 3 mm in diameter, was attached on top of the PMMA post by an acrylic solvent (Weld-On 4, IPS Corp., Gardena, CA) to keep the magnetic stir bar in position (Figure 2), thus also avoiding potential cross-talk with stir bars of neighboring microbioreactors. An additional layer of stainless steel grid (B-PMX-062, Small Parts Inc., Miami, FL) was added on the top of the aeration membrane to provide a rigid perforated upper membrane (Figure 2).

Polyethylene tubing (1/32 in. outer diameter, Becton Dickinson and Company, Franklin Lakes, NJ) was inserted into small holes punched through the PDMS with fluidic needle adapters (20 gauge, Becton Dickinson and Company) to connect to the channels (two inlets, one outlet) in the bottom of the PMMA device.

The experimental setup is shown in Figure 2. OD, pH, and DO were measured by optical sensing methods.

Batch cultures were carried out by placing the microbioreactor in an aluminum chamber, which was maintained at 30 °C by flowing heated water through its base. An external magnetic stirrer (Thermolyne, SP72725, Barnstead International, Dubuque, IA) controlled the speed of the stir bar in the microbioreactor and was placed directly below the aluminum chamber. The stirring speed of the bar during cultivation was 700 rpm. OD_{600} data for biomass determination were obtained from transmission measurements using an orange LED (Epitex L600-10V, 600 nm, Kyoto, Japan). Bifurcated optical fibers (custom-made by RoMack Fiber Optics, Williamsburg, VA) led into the chamber from both the top and the bottom and connected to LEDs and photodetectors (PDA-55, Thorlabs, Newton, NJ). Phase modulation lifetime fluorimetry was used to measure both DO and pH. The DO and pH sensors were excited with a blue-green LED (505 nm, NSPE590S, Nichia America Corporation, Mountville, PA) and a blue LED (465 nm, NSPB500S, Nichia), respectively. Excitation band-pass filters (Omega Optical XF1016 and XF1014) and emission long-pass filters (Omega Optical XF 3016 and XF 3018) separated the respective excitation and emission signals, thereby minimizing cross-excitation. All instruments were PC-controlled under a LabVIEW (National Instruments, Austin, TX) software routine, which allowed for automated and on-line measurements of OD_{600}, pH, and DO every 20 min. Following each fermentation experiment, the entire volume of the culture (150 μL) was harvested and final OD_{600} and pH values were measured. Calibration curves for

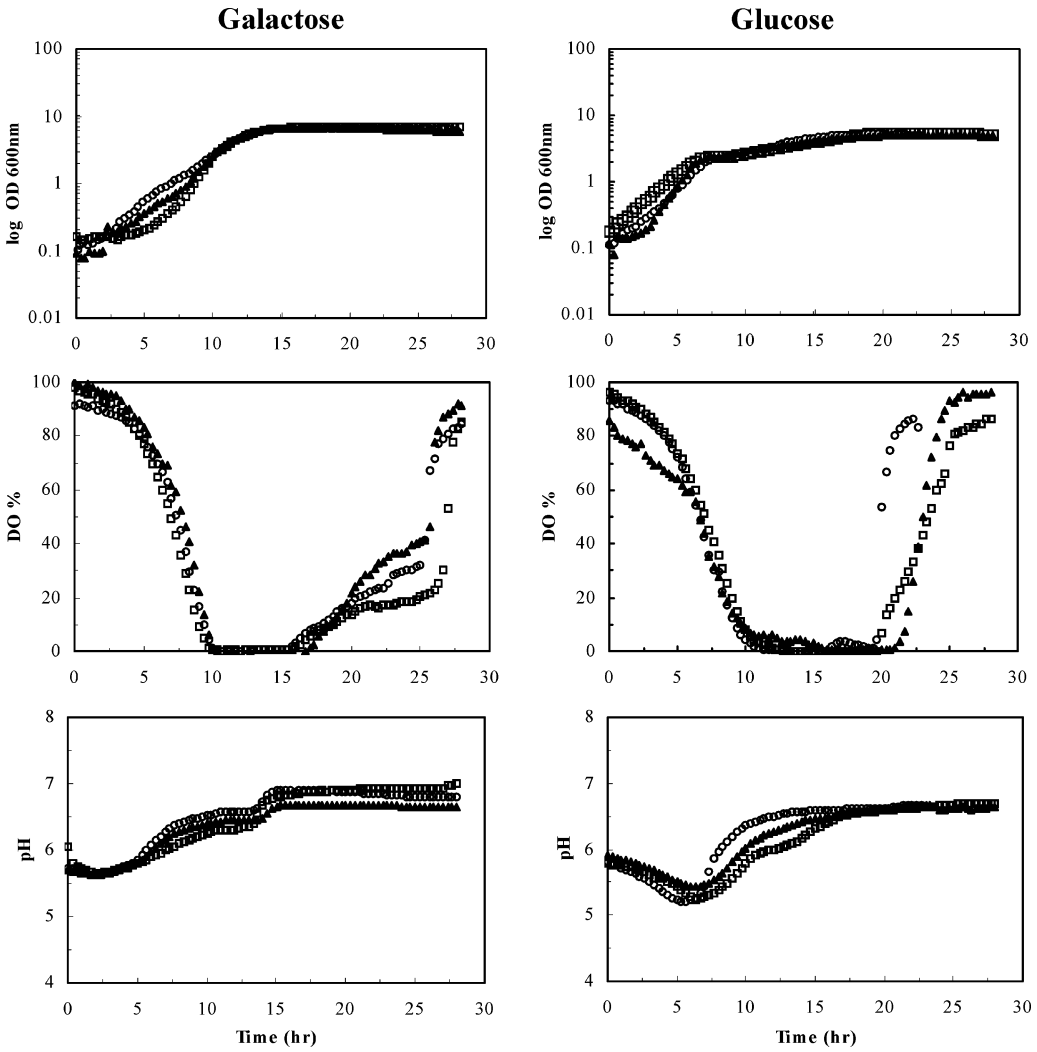

Figure 3. Batch cultures ($n = 3$) of *S. cerevisiae* grown in 150 μL stirred microbioreactors in galactose medium (left panels) and glucose medium (right panels). Growth cultures, each represented by a single data set, were performed on different days in single microbioreactors. OD_{600}, pH, and dissolved oxygen (DO) were measured in real time every 20 min.

OD readings were obtained by filling the microbioreactor with culture fluids with different biomass concentrations. The OD_{600} reading of the inoculation medium and the final OD_{600} reading were then used to calibrate the real-time OD measurements.

Each fermentation data set was obtained from an independent fermentation derived from a single colony inoculum and run in a single microbioreactor. The microbioreactor was inoculated inside the chamber by injection with a 10 mL syringe, equipped with a blunt 23-gauge flow-adaptor (Becton-Dickinson Co.), inserted into the tubing connected to an inlet side-channel. After inoculation, the inlet and outlet tubing were sealed with clamps. The chamber was then closed and real-time data collection was initiated.

One inlet channel was connected to an elevated external water reservoir to passively replenish the microbioreactor at the same rate as water evaporated through the highly permeable PDMS thin membrane, thus keeping the volume of the microbioreactor constant during fermentation. The water evaporation rate from the microbioreactor was determined to be 4.3 $\pm$0.4 μL/h at 37 °C by measuring the weight increase of water-absorbing reagent anhydrous calcium sulfate (W. A. Hammond Drierite, Xenia, OH) placed in a closed chamber with the microbioreactor. Additional details of the microbioreactor and its setup are described by Zhang et al. (2006).

Total RNA Isolation. Total RNA was isolated from two independent 150 μL batch cultures of *S. cerevisiae* grown in YPD or YPGal media. The entire volume of cultures was harvested during exponential growth, at an OD_{600} of about 1.0, through the PDMS membrane and immediately frozen in liquid nitrogen. Total RNA was isolated using an RNeasy kit (Qiagen, Valencia, CA). Cell lysis was obtained with a combination of enzymatic and mechanical disruption. Cells were pelleted and then resuspended in 100 μL of Buffer Y1 containing 100 U of lyticase (Sigma-Aldrich) and incubated for 20 min at 30 °C on a Nutator (Becton Dickinson, Parsippany, NJ) for gentle mixing. After the addition of 350 μL of buffer RLT, each sample was transferred to 2 mL tubes (Sarstedt, Newton, NC) containing 100 mg of acid-washed 425−600 μm glass beads (Sigma-Aldrich) and lysed in a FastPrep FP120 beadbeater (Qbiogene, Inc., Carlsbad, CA) for 90 s at maximum speed. The lysed samples, without the beads, were loaded directly onto RNeasy columns, and the RNA isolation was continued according to the manufacturer's protocol. The concentration and quality of the purified total RNA was assessed by determination of the OD 260/280 ratio and analysis on an Agilent 2100 Bioanalyzer (Palo Alto, CA). RNA samples were stored at −80 °C. Typical average yields of total RNA from 150 μL of *S. cerevisiae*

cultures grown to an OD_{600} of about 1 in YPD or YPGal were 7 and 5.5 μg, respectively.

Microarray Analysis. For the assessment of gene expression profiles we used Affymetrix Yeast Genome S98 Arrays (Affymetrix, Santa Clara, CA), which contain approximately 6,400 Opening Reading Frames (ORFs) of the yeast *S. cerevisiae*. Labeling and hybridization were as described in the Affymetrix GeneChip Expression Analysis Technical Manual 701021 Rev. 4 and were performed at the Whitehead Institute Center for Microarray Technology (Cambridge, MA). We carried out a total of four hybridizations, two hybridizations of two independent batch culture cultivations of YPGal grown cells and two hybridizations of two independent batch culture cultivations of YPD grown cells, starting with 5 μg of total RNA per sample. Two hundred units of SuperScript II reverse transcriptase were used for first strand cDNA synthesis reactions. Second strand DNA synthesis, cleanup of double-stranded cDNA, and biotin-labeling of cRNA were performed as described by the Affymetrix manual. The GeneChip Cleanup module was used to purify the double-stranded cDNA and the biotin-labeled cRNA. The cRNA was quantified using a NanoDrop ND-1000 UV−vis spectrophotometer (NanoDrop Tecnologies, Rockland, DE). Sample cocktails (200 μL) used for hybridizations contained 10 μg of cRNA. Microarrays were hybridized overnight, washed in a GeneChip fluidic station 450, and scanned using the GeneChip Scanner 3000 as described by Affymetrix.

Raw intensity data were imported into the S-plus Array analyzer module for normalization by Robust Multichip Analysis using GC content empirical bayes background and triple goal adjustment (GCRMA; Wu et al., 2003, 2004). Following normalization, the variance between the replicas was estabilished using the local pooled error (LPE) test (Jain et al., 2003). *P*-Values were adjusted to reflect the false discovery rate using the Benjamini−Hochberg method (Benjamini et al., 1995). Genes that differed in expression between glucose and galactose media were identified on the basis of significance ($p \leq 0.01$) and fold change ($\geq$2.0-fold). The complete datasets have been deposited in the NCBI Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/) accession number GSE2605.

Results and Discussion

Figure 3 shows growth kinetics curves with real-time measurements for OD_{600}, pH, and DO in triplicate batch cultures of *S. cerevisiae* grown in YPGal and YPD in the 150 μL stirred microbioreactors.

The entire inoculation procedure took about 25 min from the dilution of the inoculum, and this may have resulted in an increase of the lag phase of growth curves.

In YPGal medium cells reached a maximum OD_{600} of 6.87 ($\pm$0.07), and at this population density the pH and the DO concentration were 6.82 ($\pm$0.09) and 11.2 ($\pm$1.3)%, respectively, where 100% is the DO concentration of saturated medium in equilibrium with air.

In YPD medium, cells reached a maximum OD_{600} of 5.2 ($\pm$0.2) with a pH and DO concentration of 6.6 ($\pm$0.1) and 42.1 ($\pm$8.7)%, respectively (Figure 3).

The growth kinetics of *S. cerevisiae* grown in our microbioreactors were very reproducible, especially considering that individual batch cultures from independent colonies were run on different days.

In the microbioreactors, *S. cerevisiae* grew with an average maximum specific growth rate of 0.53 $\pm$ 0.02 and 0.52 $\pm$ 0.14 h^{-1} in galactose and glucose medium, respectively. In YPGal the dissolved oxygen was depleted after 10 h, approaching a concentration of 0.00 ($\pm$ 0.3)%, and it started to recover after 17 h of growth, when the oxygen concentration was 5.0 ($\pm$ 3.5)%.

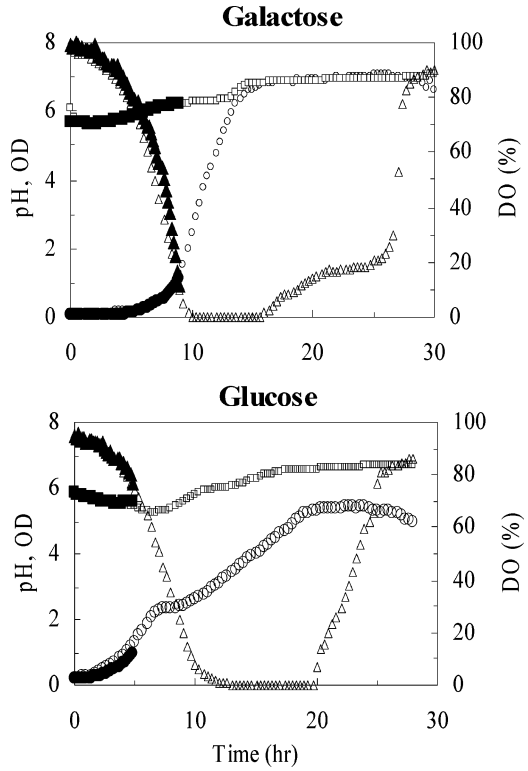

Figure 4. Reproducibility of complete batch cultures (open symbols) growth kinetics of *S. cerevisiae* grown in galactose and glucose media when cultures were sacrificed at $OD_{600} = 1$ (closed symbols). Real-time measurements of OD_{600} (circles), pH (squares), and DO (triangles) were taken every 20 minutes.

In both medium conditions, as expected, we observed a change in the growth rate of the strain when oxygen became limiting with a metabolism switch to anaerobic growth (Figure 3).

To investigate global gene expression in *S. cerevisiae* grown in our miniaturized 150 μL bioreactors, we carried out duplicate independent batch cultivation in both YPGal and YPD.

Real-time measurements of growth kinetics in the microbioreactors allowed harvesting the cells at an OD_{600} of about 1.0 for RNA isolation. Figure 4 shows the physiological status of the cells at harvesting time. At an OD_{600} of 1.0, the average pH of both media was 5.9−6.2, and the DO concentrations were on average 11.2% in YPGal and 76.7% in YPD.

To assess differential gene expression profiles under the two growth conditions, we identified upregulated genes on the basis of significance ($p = 0.01$ or less) and fold change (2.0-fold or greater).

The Affymetrix oligonucleotide GeneChip arrays (Lockhart et al., 1996) have been designed to overcome some concerns of reproducibility. Furthermore, all procedures associated with probe preparation, hybridization, and washing have been standardized (Affymetrix, 2000). For example, Piper et al. (2002) reported that reproducibility of technical replicates using Affymetrix yeast gene chip arrays was very good.

In this report the reproducibility of biological duplicates was good, and the R^2 of normalized intensities of two independent cultures grown under the same experimentalconditions and plotted against each other was above 0.97 (Figure 5).

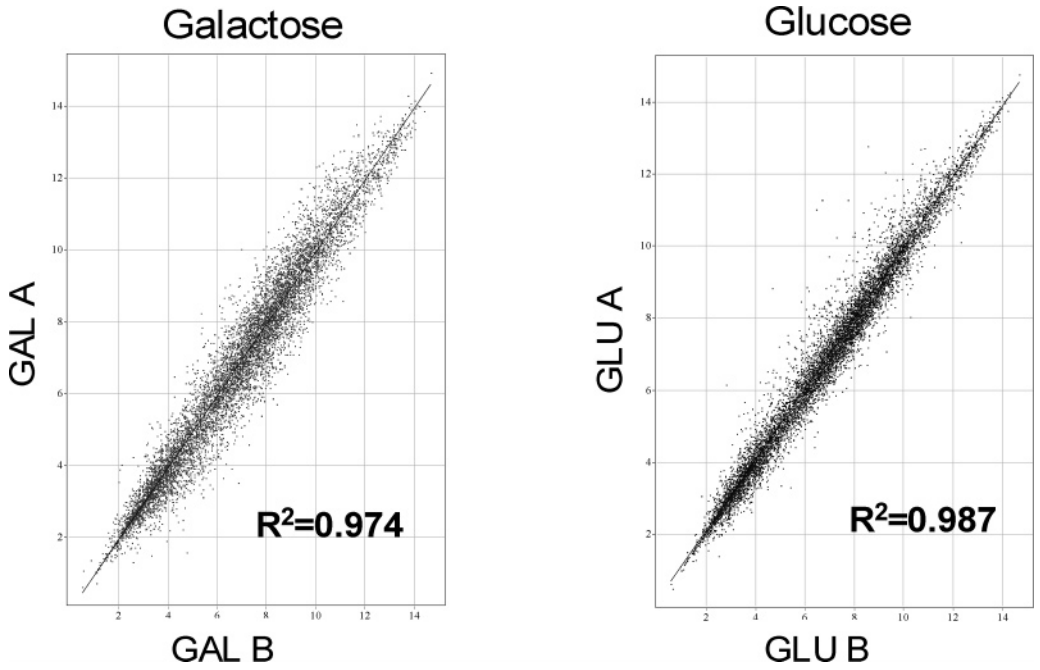

Figure 5. Correlation of microarray analysis; normalized intensities of two independent batch cultures (A and B) of *S. cerevisiae* grown in microbioreactors in galactose and glucose media plotted against each other.

Table 1. Upregulated Genes in *S. cerevisiae* Growing in Galactose Medium[a]

gene ID	gene title	Log2 GAL/GLU
GAL7	galactose-1-phosphate uridyl transferase	8.8
GAL2	galactose permease	8.7
GAL1	galactokinase	7.9
GAL10	UDP-glucose 4-epimerase	7.0
GIT1	uptake of glycerophosphoinositol (GroPIns)	4.4
DAN1	cell wall mannoprotein	3.9
GAL3	transcriptional regulator of the GAL genes	3.7
ANB1	translation initiation factor eIF-5A	3.2
PHM6	protein of unknown function	3.1
GCY1	putative NADP(+) coupled glycerol dehydrogenase	3.0
PHO84	inorganic phosphate transporter	3.0
GAL80	transcriptional regulator	2.9
SPL2	similarity to cyclin-dependent kinase inhibitors	2.8
PRM5	pheromone-regulated protein	2.4
TIR4	cell wall mannoprotein	2.4
ARR2	arsenate reductase	2.2
ICT1	protein of unknown function	2.1
PAU3	member of the seripauperin protein/gene family	2.0
IMP1	inner membrane protease	2.0
FUR4	uracil permease	1.9
PHM8	protein of unknown function	1.9
VTC3	polyphosphate synthetase (putative)	1.9
HES1	similar to human oxysterol binding protein	1.9
SSA4	HSP70 family	1.8
TIR1	cold-shock induced protein	1.8
AFR1	cytoskeletal protein|similar to arrestins	1.7
SRL3	cytoplasmic protein	1.7
CTF19	kinetochore protein	1.7
ODC1	mitochondrial 2-oxodicarboxylate transport protein	1.6
DAN3	putative cell wall protein	1.6
VTC1	*S. pombe* Nrf1p homolog	1.5
PHO5	acid phosphatase	1.0

[a] Genes were considered upregulated when the log2 ratio of galactose medium intensity over glucose medium intensity for each ORF was greater than +1.

Table 2. Upregulated Genes in *S. cerevisiae* Growing in Glucose Medium[a]

gene ID	gene title	Log2 GAL/GLU
HXT4	high affinity glucose transporter	−5.1
FDH2	NAD(+)-dependent formate dehydrogenase	−4.9
HXT2	high affinity hexose transporter-2	−4.6
FDH1	NAD(+)-dependent formate dehydrogenase	−4.0
MIG2	very similar to zinc fingers in Mig1p	−2.4
CHA1	catabolic serine (threonine) dehydratase	−2.4
STD1	MTH1 homolog	−2.2
RPL7B	ribosomal protein L7B (L6B) (rp11) (YL8)	−2.1
RPL18B	ribosomal protein L18B (rp28B)	−2.0
LTV1	protein required for viability at low temp	−2.0
ENP1	57 kDa protein with an apparent MW of 70 kDa	−2.0
RLP7	significant sequence similarity to RPL7B	−1.9
RIX1	ribosome export protein	−1.8
IMD4	IMP dehydrogenase homolog	−1.8
RCK1	serine/threonine protein kinase	−1.8
HXT1	hexose transporter	−1.8
DBP2	ATP dependent RNA helicase dead box protein	−1.8
HCA4	RNA helicase (putative)	−1.8
RPA49	RNA polymerase A 49 kDa alpha subunit	−1.7
EBP2	nucleolar protein	−1.7
NOG2	part of a pre-60S complex	−1.7
AQR1	multidrug resistance transporter	−1.7
TEF4	translation elongation factor EF-1gamma	−1.7
SUC2	invertase (sucrose hydrolyzing enzyme)	−1.7
SRP40	Nopp140 homolog	−1.7
FUR1	UPRTase	−1.7
AAH1	adenine aminohydrolase (adenine deaminase)	−1.7
NSR1	nuclear localization sequence binding protein	−1.7
NOG2	part of a pre-60S complex	−1.7
PHO87	phosphate permease	−1.7
HGH1	similarity to human HMG1 and HMG2	−1.6
HXT3	low affinity glucose transporter	−1.6
NIP7	Nip7p is required for 60S ribosomebio-genesis	−1.6

[a] Genes were considered upregulated when the log2 ratio of galactose medium intensity over glucose medium intensity for each ORF was greater than +1.

The total number of upregulated genes in *S. cerevisiae* grown in YPGal and YPD with a p-value = 0.01 was 32 (Table 1) and 33 (Table 2), respectively.

In *S. cerevisae* grown in YPGal, four genes of the galactose pathway, GAL1, GAL2, GAL7, and GAL10, were upregulated

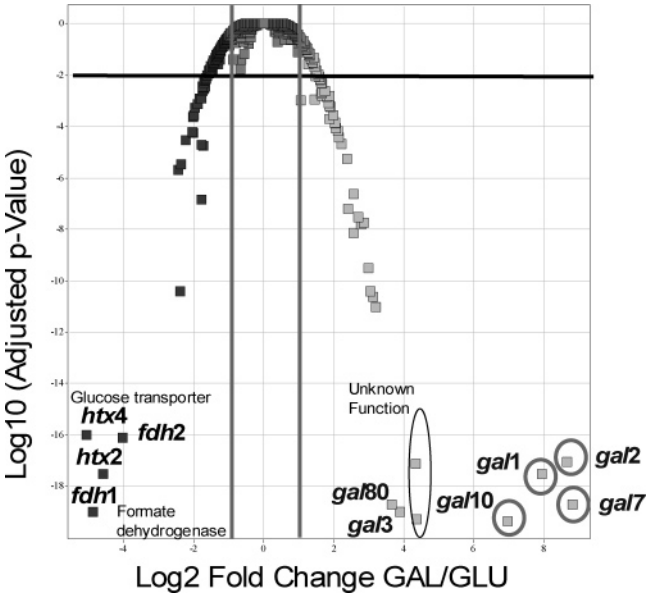

Figure 6. Volcano plot of differential gene expression in *S. cerevisiae* in galactose and glucose media. Genes were considered upregulated when the log2 ratio of galactose medium intensity over glucose medium intensity for each ORF was greater than +1 or less than −1 with a *p*-value of 0.01.

more than 125-fold (Figure 6 and Table 1). GAL5 was expressed but not found to be upregulated. This is in close agreement with previous reports that, in *S. cerevisiae* cultivated in similar growth conditions, expression of GAL5 changes only mildly or not at all (Johnston, 1987; Lohr et al., 1995). The two transcriptional regulators GAL3 and GAL80 (Figure 1) were upregulated 8- and 7.5-fold, respectively. GAL4, a third regulator, was not found to be upregulated. Similar results have been previously reported by Ideker et al. (2001).

Among the most upregulated genes in *S. cerevisiae* grown in YPD medium were two genes coding for high affinity glucose transporters, HXT4 and HXT2, whose expression increased 34.3- and 21.6-fold, respectively, and two NAD-dependent formate dehydrogenase genes, FDH2 and FDH1, whose expression increased 29.8- and 16-fold, respectively (Figure 6 and Table 2).

Conclusions

Rapid screening for microorganisms exhibiting specific patterns of gene expression and protein production is critical for progress in biological research, biotechnology, and the pharmaceutical industry. We used a microbioreactor platform, previously described by Zhang et al. (2006), that is scalable and has the advantage of providing real-time data on the microbial growth parameters OD, pH, and DO, to assess growth profiles of *S. cerevisiae* at both physiological and molecular levels.

S. cerevisiae is an extensively studied eukaryotic model system, and its galactose pathway has proven useful to evaluate our novel technologies. We have demonstrated that *S. cerevisiae* grows reproducibly in triplicate batch cultures in glucose or galactose medium in our 150 μL stirred microbioreactors. Internal mini-stirrers maintained cultures well mixed, resulting in reproducible independent microbial cultuvation data. Culture volumes were stable, indicating that the water replacement mechanism prevented water loss during cultivation. Up to the point where samples were taken, cultures in the microbioreactors that were sacrificed behaved exactly as did others that were allowed to go to growth completion. This demonstrates that (a) the collected samples were from typical cultures, and (b) the microbioreactor system is robust and reproducible.

We isolated sufficient mRNA (about 5 μg) from 150 μL of cells grown in either medium to perform Affymetrix DNA microarray hybridizations using standard protocols. Spot intensities between biological duplicate arrays of each growth condition correlated favorably.

Arrays from *S. cerevisiae* grown in galactose or glucose exhibited gene expression profiles that parallel those reported for large-scale cultures. During microbioreactor cultivation in galactose medium, the core genes of the galactose pathway, GAL1, GAL2, GAL7, and GAL10, were upregulated more than 100-fold. Furthermore, two major positive regulators of this pathway, GAL3 and GAL80, were upregulated at least 7.5-fold. Among the most upregulated genes in cultures grown in glucose medium were HTX4 and HTX2 that encode high affinity glucose transporters.

Yeast cells grown in the microbioreactors described here exhibit physiological and molecular characteristics that parallel those of large-scale cultures, demonstrating that microbioreactors are a step toward high-throughput analysis of large numbers of yeast and bacterial strains. In the future, multiplexed microbioreactors (Szita et al., 2005) could be integrated with microplate-based mRNA isolation methods such as those described by Murakami et al. (2003) to obtain high-throughput microarray analysis or with "lab on a chip" devices such as that described by Liu et al. (2004) for polymerase chain reaction amplifications and DNA microarrays.

Microbioreactors have the potential to provide much of the data and functionality that a large bioreactor system does while offering the advantages of scale for high-throughput processes.

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