Determination of elemental distribution and evaluation of elemental concentration in single *Saccharomyces cerevisiae* cells using single cell-inductively coupled plasma-mass spectrometry

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Abstract

Single-cell analysis using inductively coupled plasma mass spectrometry (SC-ICP-MS) is a method to obtain qualitative and quantitative information of the elemental content and distribution of single cells. Six intrinsic target elements were analyzed in yeast cells at different cell growth phases cultured in medium with different phosphorus concentrations (0, 7, 14 mM) to study its effect on cell growth and composition. SC-ICP-MS results were compared to those obtained by the acid digestion and the average ratio was 0.81. The limits of detection of this method were 0.08, 2.54, 12.5, 0.02, 0.02 and 0.08 fg/cell for Mg, P, K, Mn, Cu and Zn, respectively. During the exponential growth phase, the cells exhibited higher elemental contents, wider distribution for most elements and larger cell size in comparison to the stationary growth phase. Phosphorus-free conditions reduced the average P content in single cells of stationary growth phase from 650 to 80 fg. Phosphorus deficiency led to decreasing intracellular concentrations not only of P, but also of K, Cu, and to increasing Zn concentration after 48 h. Mg maintained its concentration at approximate 0.11 fg μm^{-3} and did not change significantly under the three investigated conditions after 48 h. Accordingly, Mg content was successfully used to estimate the intracellular concentration of other intrinsic elements in single yeast cells. SC-ICP-MS is suited to determine target elements in single yeast cells, allows the study of heterogeneity of cell composition and effects of stressors on the elemental content, distribution and concentrations of intrinsic elements.

Keywords

Single cell analysis, cell volume, phosphorus availability, trace element, magnesium, yeast

Introduction

A biological cell is composed of a variety of elements in various concentrations. For example, carbon is a component of many molecules and contributes to the molecular backbone of different biological macromolecules, such as proteins and polysaccharides while phosphorus can be found

in various important molecules, including the phospholipids, ATP and deoxyribonucleic acids [1, 2]. At the same time, some trace elements are present in cells with a small absolute mass, such as manganese, copper and zinc [3, 4]. The elemental contents (i.e. total mass per cell) in cells are not static but may change with the cell cycle and under stress conditions. Therefore, it gives the ability to monitor biological activities in cells. *Schizosaccharomyces pombe* and *Kluyveromyces fragilis* cells were found to have a rapid magnesium influx just before cell divisions [5]. It has also been found that nitrate-reductive environment increased the elemental contents of iron and cobalt in *Candidatus Kuenenia Stuttgartiensis* and molybdenum in *Escherichia coli* cells [6] and exposure of arsenate affected cell membrane lipids of *Chlamydomonas reinhardtii* [7].

In-depth studies of elemental cell constituents can help us gain a more comprehensive understanding of cell identification, such as cancer [8], and cellular response to external conditions [9]. Researchers have explored that Wilson's disease and cancer can induce some cells in the human body to accumulate copper [10]. With a single-cell analysis method, the difference in elemental distribution characteristics of two types of cancer cells (HeLa and A549) and normal cells (16HBE) were obtained [11]. In addition, elemental analysis of single cells showed differences in the absorption of cisplatin between sensitive and resistant cancer cells [12]. These examples illustrated the broad application prospects of studying the elemental contents in single cells.

The traditional way to obtain elemental information representing the average content of cells in a population is to digest a large known number of cells [6, 13]. With SC-ICP-MS not only the average composition of a cell culture, but the composition of single cells and their heterogeneity can be detected [14]. It is known that cells from the same population may not be identical [15-17]. These differences can be caused by factors including cell cycle, cell phenotype, and gene expression [18-22]. It is important to study these differences between individuals because it can help to improve the accuracy of results and conquer research challenges. Thus, some state-of-the-art single-cell analysis methods came into being, including SC-ICP-MS [23-25]. Detecting and quantifying the absolute mass of one element in single cells can be challenging. By virtue of high sensitivity, ICP-MS has been progressively used in the analysis of trace elements in single cells. By giving the cells one or more external labels, such as heavy metal-labeled antibodies, mass cytometry can be used to realize the characteristic analysis of single cells [26]. Recently,

the complementary use of laser ablation with the SC-ICP-MS method (LA-SC-ICP-MS) offered a high accuracy for single-cell analysis [27]. The difficulties of sample preparation and quantification are still obstacles for LA-SC-ICP-MS. Different biological cells have been analyzed for their elemental content using the SC-ICP-MS method, such as algal cells [7], bacteria [28], red blood cells [29], human cancer cells [30] and yeast [31]. Yeast is a unicellular microorganism, which is an ideal cell model for SC-ICP-MS due to its rapid growth and cell dispersion [32]. Researchers have recently studied selenium nanoparticles in yeast [31, 33] and detected the signals of several essential elements in yeast using SC-ICP-MS [34-36].

Analysis by SC-ICP-MS provides information on the mass of an element per cell, but not on elemental concentrations. This is because ICP-MS does not provide information on cell volume. For a better insight into cell physiology provision of elemental concentrations is very important, especially in phases of cell growth. Lau *et al.* reported a data plotting method, in which elemental content detected by SC-ICP-MS can be combined with cell volume information obtained by independent optical microscopy; with this approach, they calculated magnesium concentration in *Chlorella vulgaris* cells [37]. This approach opens new opportunities for applying the SC-ICP-MS method in studying elemental concentrations and dynamics in cells and allows elucidating research questions on potential parameters for cell growth and metabolic conditions.

When yeast encounters change in its growth environment, this often triggers a series of cellular responses to adapt. This affects certain cell characteristics including cell size [38]. Such cellular response may also be visible by elemental concentration in cell individuals. Therefore, in this work, SC-ICP-MS was applied and its suitability for studying elemental distributions in single yeast cells was evaluated. SC-ICP-MS was used to explore how cell growth and changes in the growth environment affect the elemental contents of yeast. The method was applied to determine the content and distribution of the six biologically relevant elements, Mg, P, K, Mn, Cu and Zn, in individual yeast cells under different growth conditions covering growth time and phosphorus availability in the medium. Complementary cell size analysis was performed to acquire correlations between cell size distribution and elemental distribution of the monitored elements, with the intention to determine a potential indicative element for the cell volume of *S. cerevisiae* under the studied conditions. Accordingly, a new approach that relies on this intrinsic element of

the cell was developed to assess the cell volume and the concentration of other intrinsic elements. Its feasibility and accuracy were evaluated.

Material & Methods

Strain and medium

S. cerevisiae H155 was obtained from the strain collection at the Helmholtz Centre for Environmental Research - UFZ (Leipzig, Germany). *S. cerevisiae* was pre-cultured in Schatzmann medium (30 g L^{-1} glucose, pH = 5.4 - 5.5). The cells were incubated for 24 h in a shaking incubator at 30 °C and 125 rpm. For the main cultivation 2 mL of 24 h pre-cultured yeast suspension was added into 1 L sterile Erlenmeyer flasks containing 200 mL of the corresponding culture medium. The optical density was measured at 600 nm (OD600, d = 5 mm) by Ultrospec 1100 pro from Amersham Biosciences (Uppsala, Sweden) [39]. To create identical inoculation for all samples, the starting OD600 value was controlled at 0.1.

The Schatzmann Medium (SM) [40], was chosen and used in this work because the elemental composition can be easily adjusted as needed. Two variations (SM-I, SM-II) with different phosphorus concentrations were prepared. The composition of the medium was listed in Table S1. SM was the control medium and contained 14 mM of phosphorus, SM-I medium contained phosphorus-reduced amounts (7 mM) and SM-II medium was free of phosphorus (0 mM).

Cell density for SC-ICP-MS analysis

Mg is one of the fundamental elements in cells and for its detection, the isotope ^{24}Mg was chosen. With increasing cell density, the number of Mg signals increased (Fig. S1). A linear relationship ($R^2 = 0.9972$) between cell density and cell signal number was determined in the range of 1 to 10×10^5 cells mL^{-1} (Fig. S2). At cell densities above 1×10^6 cells mL^{-1}, the number of cell signals did not follow the linear relationship because multiple cells, such as duplicate and triplicate cell plates, events occurred within a residence time of 3 ms. Therefore, to ensure that most of the cell signals were generated from single cells, an optimum cell density of

approximately 5×10^5 cells mL^{-1} was considered as a reference for studying elemental distribution and dynamics of the six detected elements in cells of *S. cerevisiae*.

Sampling of *S. cerevisiae*

For OD measurement and microscopy 1 mL was taken from each flask at 6 h, 24 h and 48 h. Meanwhile, 1 mL was taken for SC-ICP-MS measurement and the cells were centrifuged with 6000 g at 4 °C for 5 min (Heraeus Fresco 21, ThermoFisher, Darmstadt, Germany) to remove the medium from the cells, then washed twice with MilliQ water using the same centrifugation conditions. The MilliQ water used in the whole experiment was produced by the Millipore system of ELIX 3 combined with Element Milli-Q A10 from Merck (Darmstadt, Germany). Eventually, the cells were re-suspended in MilliQ water for SC-ICP-MS analysis within 30 min (Fig. S3). For the bulk analysis of the experiment, the cell pellets were submerged into 0.5 mL of 60 % ultra-pure concentrated nitric acid (Merck, Darmstadt, Germany) at room temperature overnight after the second washing step. The cell digestions were tested by ICP-MS using a routine quantitative method after a 70 °C water bath for 2 h.

Cell observation and microphotography

Cells of *S. cerevisiae* were observed and counted under the microscope Leica DM5500B (Leica Microsystems, Wetzlar, Germany) by C-Chip, a disposable hemocytometer (NanoEntek, South Korea). The cell suspension was diluted to obtain the relationship between OD600 values and cell density. A linear relationship was obtained ($y = 2 \times 10^7 x - 9.41 \times 10^5, R^2 = 0.9904$) (Fig. S4). To collect cell size information, the cell suspensions were diluted immediately after harvesting to an approximate cell density of 1.5×10^6 cells mL^{-1}. Three rectangular areas (about 670 × 900 μm with a chamber depth of 100 μm) were randomly selected under the microscope to take pictures. After that, photo files were stored and analyzed by ImageJ software for calculating the size of the cells for each sample. The software automatically distinguishes the cell as a particle object from the background and draws its outline to obtain a diameter value. To calculate the volume of *S. cerevisiae* cells, it was assumed that the cells after 48 h of incubation were spheres; this is confirmed by the photographs (Fig. S5).

ICP-MS and standard solution

ICP standard solutions of Mg, P, K, Mn, Cu and Zn were purchased from Merck (Darmstadt, Germany). Indium ICP standard solution originated from SPEX CertiPrep (Metuchen, NJ, USA), which was used as the internal standard. The iCAP Q's, from Thermo Fisher (Bremen, Germany), the performance was checked daily by a running tuning solution per the instructions and guidelines provided by the company. The ICP-MS instrument in this work was equipped with a quadrupole mass spectrometer, requiring multiple runs of aliquots of the same cell sample, each for one element.

The typical parameters of ICP-MS for this work were listed in Table S2. Following the publication of Liu in 2019 [35], the duration of one single yeast (commercial baking yeast) cell event in ICP-MS was accurately measured, resulting in a span from 0.5 to 0.9 ms. Therefore, 3 ms was chosen as the dwell time in this experiment. According to the manufacturer, the dead time of the instrument was 40 ns.

Quality control measures

To estimate the probability distribution of cell signals and the SC-ICP-MS methodological repeatability, three separate measurements under identical conditions were performed with the cells from one cell population. The cells were harvested after 24 h of inoculation in Schatzmann medium and prepared for SC-ICP-MS measurements. The cell density was adjusted to approximately 5×10^5 cells mL^{-1} by dilution with MilliQ water. Three separate measurements data and their sum data were fit by a lognormal distribution. An asymmetric and broad elemental distribution with a tailing towards higher Mg content in cells was observed and it was fitted with a lognormal distribution (Fig. S6). Lognormal distributions are typical for biological cells [41], however, Gaussian and Poisson distributions may also occur [11, 27]. The results of the triplicate analysis and their sum are listed in Table S3. The parameters of the triplicates, such as the signal number (RSD=2.35%) and mean intensity (RSD=3.78%), were similar, and their matched lognormal distributions were also close in the terms of log standard deviation and coefficient of determination. Cell transport efficiency (φ_{cell}) was calculated by counting the cell signal number

from SC-ICP-MS measurement compared to the total number of introduced cells within the measuring period.

Data processing

The raw data of each measurement were transferred into Excel. To distinguish cell signals from noise, any data point that was higher than the value of average plus 3 σ of the whole dataset was collected and treated as a cell signal, and the remaining data were manipulated again in the same way. All singled out data were regarded as cell signals, and the remaining data were considered as background.

According to the signal intensity of each cell, the absolute mass of an element in one single cell m_{cell} can be calculated using the equation (1) [11].

$$m_{cell} = \nu \times \tau \times \eta \times \frac{I_c - I_b}{a} \times 16.67 \quad (1)$$

Where m_{cell} stands for the mass of one element per cell event (fg/cell), ν is the sample uptake speed (mL min^{-1}), τ is dwell time (ms), η represents ICP-MS transport efficiency of a liquid standard solution, I_c and I_b are the intensity of cell signal and background (cps), respectively, a represents the slope of the calibration curve of element standard (cps/(ng mL^{-1})). The constant 16.67 is used for unit conversion between millisecond to minute and nanogram to femtogram.

Moreover, Welch's unequal variances *t*-test was performed to check if the elemental content differences of cells were statistically significant. Elemental concentrations were calculated according to the data plotting strategy reported by Lau *et al.* [37].

The distributions of each element were matched by the lognormal or Gaussian distribution. Some elemental distributions of 6 h samples possessed signal noise, as showed in Fig. S7, and the reason for that might be part of the ion cloud generated by some large budding cells being recorded as cell signals in two reading slots. Since these signals are close to zero, they were regarded as background noise rather than cell signals and were, hence, not included into further data processing.

Results & Discussion

Method performance

In ICP-MS analysis, sensitivity is element dependent. Thus, not all elemental cell constituents may be detected and quantified successfully by SC-ICP-MS. Several analytical method parameters were determined during method establishment and evaluation for describing the dynamic working range of the SC-ICP-MS method, in terms of cell signal, cell density, element sensitivity, limit of detection (LOD), limit of quantification (LOQ) (Table 1) and method repeatability.

The six elements Mg, P, K, Mn, Cu and Zn were successfully detected in cells of *S. cerevisiae* by SC-ICP-MS. The LOD ranged from 0.02 fg/cell (Mn and Cu) to 12.48 fg/cell (K) and LOQ ranged from 0.07 fg/cell (Mn and Cu) to 37.82 fg/cell (K) (Table 1). Other essential elements contained in cells (such as oxygen, nitrogen and carbon) cannot be effectively analyzed due to the excessive background: the aerosols are composed of water, and the air contains water, oxygen, and carbon dioxide. In addition, some other indispensable elements in cells are difficult to analyze due to the gas supply of ICP-MS. The argon ^{40}Ar required for the plasma and sample transportation impacts the detection of ^{40}Ca isotope and its polyatomic interference $^{40}Ar^{16}O$ affects negatively the isotope signal of ^{56}Fe. Although calcium has more than one isotope, the natural abundances of other isotopes are low and their signals were difficult to measure.

It is noted that the cell transport efficiency (φ_{cell}) of this experiment was about 0.30 % (Table S3). This is likely due to the use of a cyclonic spray chamber, where cell deposition occurred, and thereby reducing cell transport efficiency. Similar values have been reported in another study [42]. Spray chambers with a linear path specifically designed for cell suspensions were reported to increase the transport efficiency of single cells [43, 44] and may be recommended for future experiments.

SC-ICP-MS vs. elemental bulk analysis

Bulk analysis from acid digestion of a known number of cells was carried out by ICP-MS to verify the accuracy of the data obtained by the SC-ICP-MS method. The ratio between these masses ranged from 0.45 for K in the sample '6 h SM-II', to 1.51 for Zn in the sample '24 h SM-II' (Fig. 1). The average of all values was 0.81, indicating a 20% underestimation of the elemental content by the SC-ICP-MS approach. This underestimation may be due to several reasons: (i) the transport efficiency into the plasma may be lower for some larger cells; (ii) single cells may not be completely atomized and ionized in the plasma, similar to solid particles [45, 46]; (iii) the ion clouds produced by single cells might not be completely recorded by one reading slot, causing part of its signal to be included in the next reading and then treated as another cell or as background noise. Increasing the dwell time can almost ensure that the complete cell signal is in one reading slot, but it also increases the probability of multi-cell events and the intensity of noises.

Calibration of SC-ICP-MS by nanoparticle suspensions was recently reported to increase the accuracy compared to calibration with ionic solutions [45]. Moreover, polyatomic interference may result in overestimation, as found for Zn in this study (Fig. 1). Such interferences could be avoided in the bulk analysis, because this was performed using a collision cell. However, the collision cell could not be used for single-cell detection, because it smoothened and, thus, deteriorated the spike-like signals of the individual cells.

Overall, the ratio values in this experiment are comparable to other studies, for example, the ratio ranges of SC-ICP-MS results and digestion results were reported as 0.61 - 1.36 (mean = 0.98) [11], 0.59 - 2.28 (mean = 1.00) [35] and 0.23 - 1.23 (mean = 0.82) [42]. Therefore, it can be concluded that the SC-ICP-MS analysis method provided accurate results for the analysis of elemental contents in single cells of *S. cerevisiae*.

Cell number and cell size analysis

The cell density, shape and size of the cells of *S. cerevisiae* were determined by OD measurement and microscopy at three-time points for yeast cells grown in the reference medium SM and two further media, SM-I and SM-II, where phosphorus amount was half of SM and zero, respectively. In the reference medium SM, the cell density increased from $1.70 \pm 0.03 \times 10^7$ cells

mL^{-1} after 6 h of cultivation to $1.78 \pm 0.12 \times 10^8$ cells mL^{-1} after 24 h with a further increase of only 25 % at 48 h (Fig. S8). Bud shaped structures dominated at 6 h of cultivation, which were diminished at 24 h and almost completely disappeared at 48 h (Fig. S5). Single cells without bud shaped structures appeared as spheres under the microscope. The mean cell size decreased from 15 µm at 6 h to 9 µm at 48 h ($n \approx 300$ cells) (Fig. 2). Accordingly, the three sampling time points of the SM medium represented three different growth phases: the logarithmic growth phase, the beginning of stationary phase and the middle of stationary phase, for 6 h, 24 h and 48 h after inoculation, respectively.

The OD600 values, cell density and the cell size and its distribution were similar between growth on media SM and SM-I, with a slight 13 % decrease in the cell size at 24 h. For the SM-II phosphorus-free medium, marked differences were visible: OD600 values reached only half of the values of the reference medium and the cell number was only 9×10^7 cells mL^{-1} after 48 h of cultivation. Also cell sizes were significantly ($p < 0.005$) smaller (-14 % to -20 %) at all three sampling time points (Fig. 2). It can be concluded that phosphorus-free conditions inhibited the cell growth, resulting in smaller cell sizes, and this effect was also greater than the one observed in phosphorus-reduced conditions.

Phosphorus is regarded as one of the essential elements for cell growth. This element is necessary for many cellular processes, such as DNA replication, cell membrane synthesis and protein synthesis regulation [47]. Insufficient phosphorus content in the environment of cells may force the cells to use the limited intracellular phosphorus reserve until cell cycling ceases [47, 48]. Thus, in the absence of phosphorus, i.e. in the medium SM-II, cell growth was significantly suppressed, which can be inferred as part of the cellular activities being inhibited accordingly. The elements involved in these cellular activities could change on a quantitative level.

Elemental content in the cells of *S. cerevisiae*

The use of SC-ICP-MS to track changes in elemental content of individual cells can provide detailed data and information for a more in-depth explanation of how cells respond to extreme environments. For the elements, Mg, P, K, Mn, Cu and Zn in the cells of *S. cerevisiae*, elemental

contents at the three sampling points were found in different mass ranges. For example in SM medium, in the case of P, the range was from lowest 653 fg/cell (mean) at 48 h to highest 878 fg/cell (mean) at 6 h, and in the case of Cu, it ranged from 0.4 fg/cell (mean) at 6h to 1.6 fg/cell (mean) at 48 h (Table S4).

The data suggest that the elemental content of single cells of *S. cerevisiae* depends on the growth phase of the cell population (Fig. 3A). For instance, the lognormal elemental distribution of Mg changed from a maximum at approx. 90 fg/cell at 6 h to approx. 30 fg/cell at 24 and 48 h, and the distribution became narrower (Fig. 3A). It is often seen and well understood, that the difference between individuals (cells as well as organisms) during phases of rapid development are higher than during stable phases.

The elements P, K, Mn and Zn showed a similar trend (Fig. 3A). In the logarithmic growth phase, that is, 6 h after inoculation, the cells carried the highest content of these elements, which then gradually decreased towards the stationary phase (24 h and 48 h). Only Cu shows a distinctively different pattern with the lowest contents per cell during the exponential growth phase of the yeast (6 h), an increase towards 24 h with a very broad distribution and a slight decrease in the maximum at 48 h (Fig. 3A). It has been reported previously that much of the uptake of Cu by *S. cerevisiae* occurs after the early growth phase [49]. It is known that *S. cerevisiae* interacts with metal ions through the negatively charged sites of the cell surface, then the ions are internalized by transmembrane proteins [50-52]. It is, therefore, speculated that this internalization process of Cu might be slower than for other metals, such as Zn. Cu was found to have a strong ability to bind organic molecules to form complexes, which may reduce its availability to cells in the medium [53, 54].

The reduction in phosphorus availability may affect the elemental content in single cells of *S. cerevisiae*. Thus, the elemental distribution for the investigated elements was also analyzed in cells grown with less (SM-I) and no (SM-II) phosphorus in the medium. Yeast cells that were grown in the phosphorus-reduced medium SM-I (Fig. 3B) showed similar elemental distributions (Fig. 3A) and average elemental contents (Table S4) to the reference. However, some subtle differences were also visible from a reduction in the average cell size at 24 h (Fig. 2).

The phosphorus-free medium SM-II did not support the growth of *S. cerevisiae*, leading to reduced mean contents for all monitored elements (Table S4). The smallest difference was seen

for Zn with a 50 % lower level at 6 h, but almost the same level as in SM at 48 h. These lower mean values of all elements are also reflected in the elemental distributions (Fig. 3C). Contrary to the development in the phosphorus-containing media (Fig. 3A, B) elemental contents remain low from the beginning on and the final levels are also lower. For example, the maximum in the Mg content is about 20 fg/cell, compared to a maximum at 90 fg/cell in the case of SM and SM-I.

It should be noted that the cell size was also reduced under this extreme condition, therefore the concentration of Mg in the cell may not be significantly disturbed (see below). These data prove that the phosphorus-free condition affected the internalization of related elements by cells. Besides the smaller cell size, the cell surface also decreased, which might cause less binding area for ion uptake [55]. The loss of internal contents of P from earlier (6 h) to later phases of development (48 h) may be due to the transfer of an element from the parent cells to the daughter cells for their demands [48, 56].

Elemental concentration in the cells of *S. cerevisiae*

In this work, to investigate if the element contents are dependent on cell size, the cell volume was determined using cells from SM, SM-I and SM-II media at 48 h, when most cells were spherical. From a picture of the cell sample by microphotography, the software automatically recognized the cell size to obtain the diameter and estimated their volume. This was not possible in the exponential growth phase (6 h), because yeast cells are forming buds and do not exhibit a spherical shape. Therefore, only the cells at 48 h were evaluated for elemental concentration in this section.

A data plotting approach was, then, applied to link the cell size data from the microscopic analysis with the elemental content data gathered by SC-ICP-MS from the same culture to calculate elemental concentrations in single cells. This approach assumes that smaller cells contain lower elemental contents than larger cells; on this basis, the data on cell size/volume measured offline can be correlated with the elemental content in single cells [37]. Cell volume data and elemental content data were separately arranged from small to large and the 1^{st} to 100^{th} percentile data were singled out for forming a new set of data to assign the lowest elemental

content to the smallest volume subsequently till the highest elemental content to the largest volume. Afterward, these data points were used to plot the graphs of cell volume to the corresponding elemental content.

Fig. 4 shows the linear relationship between the cell volumes and the elemental contents; the slopes of the linear regression are numerically equal to the average elemental concentrations of six target elements in single cells. One candidate element as a cell volume indicator is Mn, whose three slopes almost coincided (Fig. 4). But the significant difference of Mn concentration between SM and SM-II (Table 2) suggested it cannot maintain its intracellular concentration stably. In addition, its low absolute content in a single cell may pose challenges for detection.

After 48 h of incubation, the Mg concentrations in the cells of *S. cerevisiae* remained relatively stable at approximately 0.11 fg μm^{-3} (RSD = 5%) among all three media (Table 2). Since the changes of Mg concentration in cells under phosphorus-related stress were negligible compared to the other elements, Mg may be used as an internal element indicating cell volume in these experiments with *S. cerevisiae*. Then, concentration data could be generated from the mass per cell data without the need for independent microphotography. It was previously suggested for the algae *Chlorella vulgaris* per organism could be used as a measure of algal volume [57]. For the yeast at 48 h, based on our data, this may indeed be the case. However, whether Mg can maintain its intracellular concentration under all conditions and also during earlier cell growth phases needs to be studied further.

Growth under phosphorus-free conditions (SM-II) caused the concentrations of P, K and Cu to drop and of Zn to increase in cells of *S. cerevisiae* (Fig. 4 and Table 2). Possibly, the lack of phosphorus leads to a restriction of cell growth and a decline in demand for related elements. Zn determination from the media showed that it was completely consumed by the yeast cells in all experiments during the cultivation time (Fig. S9). As cells remained lower in number at phosphorus-free conditions (SM-II), the mass per cell could increase compared to SM and SM-I.

As listed in Table S5, the average ratio of the elemental concentration values obtained by these two methods, data plotting and acid digestion, is 0.84. For the masses, the ratio was 0.81 (see above). Thus, the conversion of mass into concentrations does not introduce an additional systematic error. This approach of converting mass per cell data into concentration in cell data based on cell volume determination from microphotographs may even be extended to non-

spherical cells occurring in the early growth phase, provided that 3D imaging is applied. With this correlation approach, however, inter-cellular differences in elemental concentration cannot be detected. Rather, the approach assumes an average concentration per cell (slopes in Fig. 4), while in reality individual smaller cells may exhibit higher elemental concentrations than larger cells. To account for such inter-cellular concentration differences cell volume detection on-line with SC-ICP-MS would be required. This would allow to link the volume information to the elemental content information for each single cell.

Determination of elemental concentration using Mg as a volume indicator

It was then tested whether the Mg mass can be used as an indicator for yeast cell volume at 48 h under the studied conditions. For this purpose, a Mg concentration of 0.11 fg μm^{-3} was used to calculate the concentrations of the other five elements based on Mg mass in single cells. As the quadrupole-MS required independent runs to determine the mass distribution of each of the elements, a correlation between the Mg mass and the mass of each of the elements had to be established. For that purpose, the same correlation approach as used before was applied: mass data obtained for each of the elements were ordered from low to high mass and it was assumed that the 1^{st} percentile of mass data for Mg is related to those yeast cells that exhibit the 1^{st} percentile of the mass of any other of the five elements, and so on up to the 100^{th} percentile. Equation (2) is used to calculate the concentration of the other five elements (P, K, Mn, Cu and Zn) in single cells of *S. cerevisiae*.

$$c = \frac{m \times c'}{m'} \quad (2)$$

Where *c* stands for elemental concentration in yeast cells, *m* represents elemental content, *c'* and *m'* are elemental concentration and elemental content of Mg.

The mean concentrations of the five elements in yeast cells of the three growth experiments compare well with the concentration data calculated from the volume information gathered by using the microphotograph data (Fig. 5). For Mn, the difference between the two approaches appears to be a bit larger (24%-113%) than for the other elements. This may be due to a higher measurement uncertainty for Mn as the detected masses were close to the LOD.

For this set of experiments, it appears that Mg mass is an appropriate indicator for the cell volume of *S. cerevisiae* and can, therefore, be used to calculate concentrations of other intrinsic elements from the mass data determined by SC-ICP-MS. The option to use the mass of one element as an indicator of cell volume would be very advantageous for several reasons: (i) it would replace independent and time-consuming cell size distribution analyses, which estimate the cell volume from microscopic observation by a simple analysis of an appropriate element by the established SC-ICP-MS approach; (ii) it can also avoid systematic errors caused by analyzing and estimating the volume of individual cells through photos from the microscope.

However, the applied correlation approach assumes that a yeast cell with a low Mg mass also contains lower masses of the other elements, this assumption may not be true for all individual cells. The use of time-of-flight mass spectrometer to SC-ICP-MS (SC-ICP-TOF-MS) would not have to rely on such assumptions but would enable determining also concentration differences for different elements in individual cells, provided that one of the elements would exhibit a stable concentration. The availability of such instruments is, however, limited.

Conclusions

The conventional methods of determining the contents of elements in cells from digested samples cannot provide information on the contents of individual cells and differences among them. Single-cell analysis can reveal cell heterogeneity. In this study, elemental contents in the order of femtogram and even attogram in a single cell were monitored and quantified by SC-ICP-MS.

The SC-ICP-MS method applied in this experiment revealed changes in the elemental composition of yeast cells during growth. Single cells contained a higher elemental content of Mg, P, K, Mn and Zn and a lower of Cu in the early growth phase (6 h) compared to cells in stationary phase (24 h and 48 h). Changes in elemental contents over time can potentially indicate an internalization process. Stress during growth by P-deficiency led to less elemental contents for almost all target elements in the yeast cells. This is the first time that the dynamics of intrinsic elements in single yeast cells have been studied by SC-ICP-MS under the conditions of the different growth phases and phosphorus availability.

For the yeast cells at 48 h the Mg concentration in cells remained relatively constant. Therefore, the Mg content was used as an indicator for cell volume to estimate the concentrations of other intrinsic elements from their respective mass more accurately. For the cells of *S. cerevisiae* under different growth conditions and growth phases, and for other types of cells, the indicator element for cell volume needs to be further investigated. Anyhow, the strategy of using one intracellular element as an indicator of cell volume and, thus, to convert elemental contents into elemental concentrations solely on the basis of SC-ICP-MS measurements appears promising.

A closer insight into individual elemental concentrations of multiple elements will be provided by SC-ICP-TOF-MS. Along with the improvement of the cell transport efficiency, this will further expand the potential of SC-ICP-MS for elemental analysis in single cells.

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Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by WQ with the support of H-JS. The first draft of the manuscript was written by WQ and all authors contributed to manuscript elaboration. All authors read and approved the final manuscript.

Conflict of Interest

There is no conflict to declare.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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