A novel approach to regulate cell membrane permeability for ATP and NADH formation in *Saccharomyces cerevisiae* induced by air cold plasma

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Abstract
Air cold plasma has been used as a novel method for enhancing microbial fermentation. The aim of this work was to explore the effect of plasma on membrane permeability and the formation of ATP and NADH in *Saccharomyces cerevisiae*, so as to provide valuable information for large-scale application of plasma in the fermentation industry. Suspensions of *S. cerevisiae* cells were exposed to air cold plasma for 0, 1, 2, 3, 4 and 5 min, and then subjected to various analyses prior to fermentation (0 h) and at the 9 and 21 h stages of fermentation. Compared with non-exposed cells, cells exposed to plasma for 1 min exhibited a marked increase in cytoplasmic free Ca²⁺ concentration as a result of the significant increase in membrane potential prior to fermentation. At the same time, the ATP level in the cell suspension decreased by about 40%, resulting in a reduction of about 60% in NADH prior to culturing. However, the levels of ATP and NADH in the culture at the 9 and 21 h fermentation stages were different from the level at 0 h. Taken together, the results indicated that exposure of *S. cerevisiae* to air cold plasma could increase its cytoplasmic free Ca²⁺ concentration by improving the cell membrane potential, consequently leading to changes in ATP and NADH levels.

Keywords: *Saccharomyces cerevisiae*, air cold plasma discharge, membrane permeability

(Some figures may appear in colour only in the online journal)

1. Introduction

Cell membranes not only determine cell shape and provide protection for the cell but they are also involved in metabolic processes, such as the transport of ions and nutrients, the storage and transmission of energy, as well as in cell signaling and communication [1]. During biotransformation, the rates of nutrient uptake and release of metabolic products are greatly restricted by the cell membrane. Therefore, the production capacity of microbial cells in biocatalysis and fermentation may be gravely compromised [2]. It is therefore necessary to artificially regulate cell membrane permeability to improve the yield of target products in a biotransformation process.

Numerous pre-treatment technologies have been developed to control the membrane permeability of microbes. These technologies include osmotic pressure, oxidative stress and exposure to rare-earth ions, electric fields and microwave irradiation [3–7], and they work by altering the functional properties of the cell membrane to promote permeability and the transport of ions and macromolecules. However, these methods have several drawbacks. For example, chemical methods such as osmotic pressure, oxidative stress and rare-earth ion exposure, generate enormous amounts of hazardous waste, while physical methods, such as electric field and microwave irradiation, are difficult to apply at large scales.
Therefore, there is a need to develop a novel approach for altering cell membrane permeability, one that is simple and has superior efficiency in addition to being an energy saving and environmentally friendly process. Such a method would have great benefit for the environment and wide market appeal.

Air cold plasma has recently been recognized as a novel and advantageous pre-treatment technology because of its high efficiency, low energy consumption and environmentally friendly features. In recent years air cold plasma has been widely used in sterilization and mutagenesis of industrial microbes as a result of its physical and chemical properties [8–10]. In our earlier studies, we reported that dielectric barrier discharge (DBD) plasma at air atmospheric pressure can improve the productivity of 1,3-propanediol in Klebsiella pneumoniae in batch fermentation by about 56% [11]. The cell membrane permeability of the exposed cells is also markedly increased throughout the fermentation process [11]. Furthermore, the ethanol yield from plasma-exposed Saccharomyces cerevisiae has a close relationship with the plasma parameters, such as plasma discharge time, power voltage and the volume of the cell suspension used in the exposure. Under the conditions of 1 min, 26 V and 9 ml, the final yield of ethanol is 0.48 g g$^{-1}$, an increase of 33% over the control [12].

Bakers’ yeast, S. cerevisiae, is widely used in the biological production of ethanol. Increasing the permeability of the yeast cell membrane would promote the rates of glucose uptake and utilization, thereby increasing the ethanol yield in the fermentation. The glucose metabolic pathway of S. cerevisiae during anaerobic fermentation is shown in figure 1. The tricarboxylic acid cycle (TCA) pathway does not operate as a cycle in the mitochondrion, as most of the earlier reports have described, but, rather, it occurs as two branches in the cytosol [13].

Cofactors such as adenosine triphosphate (ATP) and the reduced form of nicotinamide adenine dinucleotide (NADH) play important roles in the distribution and rate of metabolic fluxes in a metabolic pathway. ATP is obtained from glycolysis, and its increase may enhance the growth rate of the cell or organism [14]. However, Larsson et al reported a strong inverse proportional relationship between the intracellular ATP level and the rate of glycolysis; i.e. the lower the concentration of ATP, the higher the glycolysis rate [15]. The rapid consumption of glucose could lead to the accumulation of NADH. Most of the NADH produced during glycolysis is subsequently oxidized and used in ethanol synthesis.

The calcium ion is an important secondary messenger. Changes in cytosolic Ca$^{2+}$ concentration are associated with the regulation of an extensive variety of metabolic reactions, such as cell metabolism, cell signaling, membrane transport channels and the activities of some types of ATPases [16]. As shown in figure 1, an increase in the Ca$^{2+}$ concentration in the cytoplasm can be the result of increased inflow of extracellular Ca$^{2+}$ through the cell membrane-located Cch1 protein/Mid1 protein (Cch1p/Mid1p) channel or as a result of outflow of vacuolar Ca$^{2+}$ into the cytoplasm via the Yvc1 protein (Yvc1p) channel on the vacuole membrane [17–20]. The opening of an ion channel is one of the mechanisms by which cell membrane permeability is enhanced. Up to now, little information has been available on the impact of air cold plasma on cell membrane permeability and the levels of cofactors in yeast metabolism during ethanol fermentation.

The aim of the present study was to explore the influence of exposure time to air cold plasma on the permeability of the yeast cell membrane by measuring the changes in cytoplasmic free Ca$^{2+}$ concentration after subjecting cells to different plasma exposure times. In addition, changes in the levels of the cofactors, ATP and NADH, were also measured to investigate the effects of plasma exposure time on cofactor metabolism. This study aimed to provide useful information concerning the possible use of air cold plasma to control the metabolism of cofactors in yeast cells by altering cell membrane permeability.

2. Experiment

2.1. Microorganism and growth condition

Yeast strain S. cerevisiae CGMCC no. 6184 was obtained from the China General Microbiological Culture Collection Center (CGMCC, Beijing, China).

Yeast cells were grown in yeast extract peptone dextrose (YPD) solid medium (yeast extract 10 g l$^{-1}$, glucose 20 g l$^{-1}$, peptone 20 g l$^{-1}$, agar 20 g l$^{-1}$) for strain preservation and viable count assay. YPD liquid medium was used for general
cell propagation whereas YPD liquid medium containing 180 g l\(^{-1}\) glucose instead of 20 g l\(^{-1}\) was used for fermentation.

2.2. Preparation of a pre-exposed sample

Yeast CGMCC-6184 preserved in a refrigerator was revived on YPD agar medium at 28 °C for 36 h. After that, one loop of yeast cells was inoculated into 100 ml of YPD liquid medium and incubated on a rotary shaker at 200 rpm and 28 °C. The cells were harvested in the logarithmic phase by centrifugation at 12 000 \(g\) and 4 °C for 15 min. Finally, the cell pellet was suspended in sterile YPD liquid medium to an optical density of 600 nm \((\text{OD}_{600})\) of 10 (about 1.0 \(\times\) 10\(^6\) cells ml\(^{-1}\)) for plasma exposure.

2.3. Cold plasma discharge processing

The cold plasma discharge equipment and the main steps involved in the sample exposure process using cold plasma (figure 2) were similar to those described in our earlier report [21]. The unexposed and exposed cell suspensions were immediately subjected to further analyses, as described below, or to flask fermentation. Samples from a fermentation culture were withdrawn at 9 and 21 h for the same series of analyses. A more detailed description can be found in a previous report [21].

2.4. Determination of membrane permeabilization

Membrane permeabilities of unexposed cells and cells exposed to plasma were determined at the 0, 9 and 21 h culturing stages, as described previously [21], but with modifications. Fluorescein diacetate (FDA) was added to the yeast suspension to a final concentration of 0.5 mg ml\(^{-1}\). After incubation in the dark at 37 °C for 5 min, the cells were collected, washed twice in phosphate-buffered saline (PBS) and then re-suspended in PBS to a density of 1.0 \(\times\) 10\(^6\) cells ml\(^{-1}\). The fluorescence of the cells was measured with a fluorescence spectrophotometer (JASCO Japan Co., Ltd). Excitation and emission wavelengths were set at 269 and 517 nm, respectively. The fluorescence intensity of the sample was expressed as a percentage relative to the control.

2.5. Determination of the membrane potential

The membrane potential of yeast cells was measured with a fluorospectrophotometer as described previously [21, 22]. Rhodamine123 (Rh123) was added to the cell suspension to a final concentration of 10 \(\mu\)gm l\(^{-1}\). After incubation in the dark at 37 °C for 30 min, the cells were collected, washed twice and re-suspended in PBS to a concentration of 1.0 \(\times\) 10\(^6\) cells ml\(^{-1}\). The cell suspension was subjected to membrane potential measurement. Excitation and emission wavelengths were set at 244 and 486 nm, respectively. Fluorescence intensity was expressed as a percentage relative to the control.

2.6. Determination of cytoplasmic Ca\(^{2+}\)

The pre-treatment for Ca\(^{2+}\) permeability with Fluo-3 AM as the fluorescent probe was similar to the reported approach [21, 23]. Briefly, fluorescent probe Fluo-3 AM was added to a suspension of yeast cells to a final concentration of 0.5 \(\mu\)mol ml\(^{-1}\). The cell suspension was cultured in the dark at 37 °C for 30 min, followed by centrifugation at 12 000g for 15 min. The cells were washed and re-suspended in PBS. The amount of Ca\(^{2+}\) in the cytoplasm was obtained from the fluorescence intensity measured with a fluorospectrophotometer at excitation and emission wavelengths of 506 and 526 nm, respectively. The intensity was expressed as a percentage relative to the control.

Figure 2. Schematic representation showing the essential features of an air cold plasma discharge setup. (a) The protocol for enhancing membrane permeability in flask liquid culture (b).
2.7. Measurements of extracellular ATP and NADH content

The cellular content of NADH and ATP may be released into the extracellular space by active exocytosis or diffusion across membrane channels under conditions of cell stress [24]. Therefore, the supernatants of the unexposed cell suspension and cell suspension that had been exposed to cold plasma collected after centrifugation were directly used for ATP and NADH assays. ATP assay was performed with an ATP Colorimetric Assay Kit (NJCBIO, China) according to the manufacturer’s instructions. NADH assay was performed using an Amplitex™ Colorimetric Total nicotinamide adenine (NAD) and NADH Assay Kit ‘Blue Color’ (AAT Bioquest Inc., Sunnyvale, CA, USA) according to the manufacturer’s instructions.

2.8. Protein assay

The protein concentration of the above supernatants was measured using the Bradford assay [25].

2.9. Data analysis

All the measurements were performed in triplicate. All the data statistics, including means, standard errors and significance comparison, were calculated using Origin 7.0 software. Significant differences between test samples and controls were considered at the $P < 0.05$, $P < 0.005$ or $P < 0.001$ level.

3. Results

3.1. Plasma membrane permeability

The changes in membrane permeability exhibited by *S. cerevisiae* cells following their exposure to plasma and subsequent culturing under fermentation conditions are shown in table 1. After exposure to plasma for 1 min, the membrane permeability decreased compared with that of unexposed cells, but increased when the exposure time was increased from 2 to 4 min, and then fell back to the level of unexposed cells when the exposure time was increased to 5 min. The membrane permeability of the exposed cells reached a maximum when the cells exposed for 4 min were cultured for 9 h, yielding a 1.2-fold increase over that of unexposed cells. As for cells that were cultured for 21 h, a significant increase in membrane permeability only occurred for those that were derived from cells exposed to plasma for 1 and 5 min.

3.2. Plasma membrane potential

The membrane potential was detected with the aid of the fluorescence probe Rh123 (table 2). The fluorescence intensity of Rh123 was positively correlated with plasma membrane potential. These data showed that the plasma membrane potential was raised (20%) when the cells were exposed for 1 min but was reduced when they were exposed for 2–5 min. When the exposed cells were cultured for 9 h, only the membrane potential of the cells derived from those exposed for 1 min decreased relative to those derived from non-exposed cells. Other exposure times gave different increases in membrane potential, among which 2 min exposure yielded the maximum increase (70%) over non-exposed cells. In the case of 21 h fermentation, 4 and 5 min exposure gave significant increases in membrane potential compared with non-exposure. These data seemed to indicate that cold air plasma discharge could either enhance or reduce the plasma membrane potential of *S. cerevisiae* cells.

### Table 1. Effect of plasma exposure on fluorescence intensity of FDA before and after fermentation.

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Original period of culture (0 h)</th>
<th>Middle period of culture (9 h)</th>
<th>Final period of culture (21 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
</tr>
<tr>
<td>1 min</td>
<td>87 ± 3.1b</td>
<td>100 ± 3.0</td>
<td>119 ± 4.2b</td>
</tr>
<tr>
<td>2 min</td>
<td>115 ± 3.8b</td>
<td>94 ± 4.0</td>
<td>102 ± 5.0</td>
</tr>
<tr>
<td>3 min</td>
<td>125 ± 5.0c</td>
<td>112 ± 5.9a</td>
<td>101 ± 4.6</td>
</tr>
<tr>
<td>4 min</td>
<td>127 ± 4.0c</td>
<td>219 ± 9.3c</td>
<td>97 ± 4.5</td>
</tr>
<tr>
<td>5 min</td>
<td>103 ± 2.9</td>
<td>104 ± 4.7</td>
<td>115 ± 5.9a</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SES. ‘a’, ‘b’ and ‘c’ indicate $P < 0.05$, $P < 0.005$ and $P < 0.001$, respectively.

### Table 2. Effect of plasma exposure on fluorescence intensity of Rh123 before and after fermentation.

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Original period of culture (0 h)</th>
<th>Middle period of culture (9 h)</th>
<th>Final period of culture (21 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
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<tr>
<td>1 min</td>
<td>120 ± 5.3b</td>
<td>93 ± 3.0a</td>
<td>106 ± 4.0</td>
</tr>
<tr>
<td>2 min</td>
<td>76 ± 4.0c</td>
<td>170 ± 6.3c</td>
<td>96 ± 4.9</td>
</tr>
<tr>
<td>3 min</td>
<td>67 ± 3.2c</td>
<td>129 ± 5.9b</td>
<td>108 ± 5.3</td>
</tr>
<tr>
<td>4 min</td>
<td>74 ± 3.0c</td>
<td>140 ± 6.4c</td>
<td>127 ± 6.5b</td>
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<tr>
<td>5 min</td>
<td>86 ± 3.9b</td>
<td>105 ± 5.0</td>
<td>113 ± 5.0a</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SES. ‘a’, ‘b’ and ‘c’ indicate $P < 0.05$, $P < 0.005$ and $P < 0.001$, respectively.

### Table 3. Effect of plasma exposure on fluorescence intensity of Fluo-3 AM before and after fermentation.

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Original period of culture (0 h)</th>
<th>Middle period of culture (9 h)</th>
<th>Final period of culture (21 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
</tr>
<tr>
<td>1 min</td>
<td>107 ± 1.2c</td>
<td>106 ± 1.3c</td>
<td>103 ± 4.0</td>
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<tr>
<td>2 min</td>
<td>110 ± 4.0b</td>
<td>108 ± 2.9b</td>
<td>102 ± 4.6</td>
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<tr>
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<td>115 ± 5.0b</td>
<td>96 ± 3.0</td>
<td>98 ± 3.7</td>
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<tr>
<td>4 min</td>
<td>117 ± 5.3b</td>
<td>59 ± 2.4c</td>
<td>95 ± 3.6</td>
</tr>
<tr>
<td>5 min</td>
<td>136 ± 4.8c</td>
<td>88 ± 2.0b</td>
<td>102 ± 5.2</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SES. ‘a’, ‘b’ and ‘c’ indicate $P < 0.05$, $P < 0.005$ and $P < 0.001$, respectively.
3.3. Cytoplasmic calcium content

The intracellular calcium concentration of plasma-exposed cells was measured with the fluorescence probe Fluo-3 AM (table 3). The calcium concentration in the cytoplasm increased with plasma exposure time, with 5 min exposure giving the highest increase, about 36% more than the concentration detected in the non-exposed cells. After 9 h of fermentation, cytoplasmic Ca\(^{2+}\) concentrations were markedly raised in the case of 1 and 2 min plasma exposure compared with no plasma exposure, but in the case of 3–5 min plasma exposure, cytoplasmic Ca\(^{2+}\) concentrations were lower compared with no plasma exposure.

3.4. Extracellular ATP concentration

The effect of plasma exposure on extracellular ATP concentration was most dramatic prior to fermentation (0 h) and at the 9 h stage of fermentation following plasma exposure (figure 3). Prior to fermentation, some significant decreases in extracellular ATP concentration were detected when S. cerevisiae cells were exposed to plasma for 1 and 2 min, but the significant increases in ATP concentration occurred when the cells were exposed to plasma for 3–5 min compared with non-exposed cells. At the 21 h stage of fermentation, however, the extracellular ATP concentration for 1 and 5 min exposure appeared to be somewhat lower than that of non-exposed cells. The result, therefore, indicated that plasma exposure might alter the concentration of extracellular ATP either immediately after treatment or when the treated cells are allowed to propagate for a moderate period of time under normal culture conditions.

3.5. Extracellular NADH concentration

Differences in extracellular NADH concentration between non-exposed and plasma-exposed S. cerevisiae cells were less uniform for all the three stages of testing. The differences were more pronounced between non-exposed cells and exposed cells prior to fermentation or at the 21 h stage of fermentation (figure 4). Prior to fermentation, 1 min treatment induced a decrease of 60%, but 2 and 3 min treatments caused 0.8- and 1.8-fold increases, respectively, in extracellular NADH concentration. At the 9 h fermentation stage, the extracellular NADH concentrations of exposed cells were either similar to or significantly lower than those of non-exposed cells. However, cells that were exposed to plasma for 1 min exhibited a significantly higher extracellular NADH concentration than non-exposed cells at the 21 h fermentation stage, although it remained much lower than that of non-exposed cells in the other two stages (0 and 9 h). In addition, cells exposed for 5 min also showed significantly higher extracellular NADH concentration than non-exposed cells at the 21 h fermentation stage. Taken together, these results suggest that plasma exposure can alter the extracellular concentration of NADH, either immediately after treatment or in subsequent fermentation, depending on the exposure time.

4. Discussion

In this study we have demonstrated that noticeable increases in membrane permeability of live cells were evident after the cells were exposed to plasma for 1 min (table 1). At the 9 and 21 h stages of fermentation, the membrane permeability was reduced, indicating that the influence of air cold plasma on membrane permeabilization was temporary and non-inheritable. Our result was in accordance with the finding of Yonson et al, who reported that cell membrane permeability is temporally enhanced by a miniature atmospheric pressure glow discharge plasma torch [26].

Membrane potential is a key factor in cellular functions such as signaling and transport, which can ultimately affect cell metabolism [27]. A change in membrane potential can be positively detected by a change in fluorescence intensity of an appropriate fluorophore, such as Rh123. When a discharge plasma occurs over the solution surface, a variety of physical...
and/or chemical processes are initiated. Many active species such as oxygen, hydrogen, hydroxyl and hydroperoxyl radicals are generated. These reactive species can diffuse in the surrounding liquid and induce the redistribution of charges on the inner and outer surface of the cell membrane, resulting in an increment in or reduction of membrane potential. Such alteration of the membrane potential would directly influence the plasma membrane permeability. When S. cerevisiae cells were exposed to air cold plasma, the change in the membrane potential immediately after treatment contrasted with the change in membrane penetrability (table 2 versus table 1). The cell membrane was depolarized due to the lowered potential, ultimately enhancing the permeability of the membrane. More inorganic and organic ions can then pass freely through the cell membrane as a result of this enhanced permeability [28]. After the 9 and 21 h stages of fermentation, the increment in membrane potential caused membrane hyperpolarization, and consequently increased the membrane permeability.

The alteration of cell membrane potential could activate the voltage-dependent Chh1p channel, leading to more influx of Ca2+ from the extracellular environment into the cytoplasm (figure 1). Thus the calcium level in the cytoplasm of exposed cells was increased after exposure to plasma. Air cold plasma slightly increased the cytoplasmic calcium concentration of the cells following exposure for 1 min. This might result from the increase in plasma membrane potential (table 2 versus table 3, at 0 h culture), leading to cell membrane hyperpolarization and opening of Ca2+ channels. However, the opening of Ca2+ channels did not lead to an increment in cell membrane permeability (table 1). This result suggests that the increase in cell membrane permeability might be governed by more than one channel modulator.

The trend associated with the changes in ATP content in the case of plasma exposure was different from that associated with the changes in membrane permeability with respect to exposure time. This indicates that alteration of extracellular ATP content is a direct consequence of changes in intracellular ATP. Prior to fermentation, the lower levels of ATP at 1 and 2 min plasma exposure might be due to their 6.8% and 10% increases in calcium concentration, respectively. The increased calcium concentration promoted the hydrolysis of ATP to adenosine diphosphate (ADP) (figure 3). A Ca2+ concentration gradient from 1 to 10 μM, could improve the cell function that regulates cell growth and metabolism to ultimately enhance microbial productivity. However, the high levels of intracellular Ca2+ can cause cell injury or death [29, 30]. The higher levels of ATP in the cells exposed to plasma for 3–5 min might be due to an inhibition of ATP hydrolysis caused by the higher cytoplasmic calcium concentration (table 3 and figure 3). In addition, any disturbance in environmental conditions would affect the activities of catabolic enzymes, thereby accelerating the accumulation of ATP or ADP [30]. Air cold plasma might cause the accumulation of ADP in the exposed cells within 1–2 min of exposure, and of ATP in the exposed cells within 3–5 min of exposure, as suggested by the data in figure 3. The accumulation of ATP or ADP might have instantly influenced the glycolysis rate [31], producing still different ATP concentrations at the 9 or 21 h stage of fermentation, depending on the plasma exposure time (figure 3). Air cold plasma generates various reactive species in the gas phase [32]. These active species further react with water and produce a variety of biologically active reactive species (RS) in the liquid phase, including long-lifetime RS (hydrogen peroxide, ozone and nitrate ions) and short-lived RS (hydroxyl radicals, superoxide and singlet oxygen) [33]. In our study, these reactive species could raise or lower the cell membrane potential and activate Ca2+ channels, consequently increasing [Ca2+]cyr (tables 2 and 3, at the beginning of culture). Ca2+ supplementations of 0.5 and 1.5 mM have been shown to induce the increase in ATPase activity [34]. The enhanced ATPase activity would then promote the generation of proton motive force through hydrolysis of ATP [34, 35]. A decrease in the intracellular ATP level can result in the up-regulation of the activities of phosphofructokinase (PFK) and
exposure was decreased compared with the control because of the oxidation of NADH to NAD. The oxidation of NADH to NAD is catalyzed by alcohol dehydrogenase, a key enzyme in the glycolytic pathway, leading to reduced glycerol production and eventually resulting in more carbon flux from glycolysis being funneled to ethanol [12, 37, 38].

5. Conclusion

The potential mechanism by which air cold plasma induces changes in cofactor metabolism of S. cerevisiae was investigated in this study by analyzing the alterations in plasma membrane potential, cytoplasmic calcium concentration and the two cofactors ATP and NADH. Cells exposed to plasma for 1 min showed a marked increase in plasma membrane potential, whereas cells exposed to plasma for 2–5 min showed notable decreases in plasma membrane potential. Furthermore, the concentrations of Ca^{2+} for cells exposed to plasma for 1–5 min were noticeably increased before the start of the culture compared with those of unexposed cells. The 7.0% increase in calcium concentration caused significant decreases in ATP (40%) and NADH (60%) in cells exposed to plasma for 1 min. At 9 h fermentation, the ATP content in cells derived from those exposed to plasma for 1 min increased by 72% whereas NADH content decreased by 88% relative to the control. Taken together, the mechanism by which plasma induced changes in cofactor level in S. cerevisiae appeared to be through raising the plasma membrane potential, which then led to increases in cytosolic free Ca^{2+} concentrations within the cells, ultimately improving microbial productivity. This may have an underlying and broad application in enhancing the bioconversion capability of microbes in the future.

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