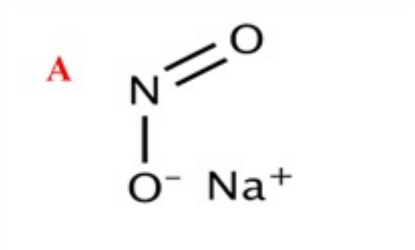
Chapter 1

Determination of the cell viability and growth of *Saccharomyces cerevisiae* under nitrosative stress

Introduction:

Reactive nitrogen species (RNS) including NO can effect on physiological and physicochemical properties of the cell [1]. These may create a hostile condition generated inside the cell, known as nitrosative stress [2]. RNS is generated inside the cell by the reaction of ROS with NO [3]. Within the solution, NO can be donated by some of the chemical species, known as NO donors e.g. acidified sodium nitrite, *S*-nitrosoglutathione, DetaNONOate, peroxynitrite etc. Each of the NO donors is different from another in respect to chemical reactivity, stability etc. [4]. Some of the compounds need enzymatic action to release NO while some other compounds produce NO non-enzymatically like through the reaction of metals, thiols etc. [5]. The percentage of NO production varies with the chemical species due to their chemical organizations like presence of non-ionic bond, covalent bond etc. Solubility, half-life, pH, light can also affect the stability and the kinetics or production of NO from NO donors [6]. NO donors like acidified sodium nitrite (ac.$NaNO_2$) and *S*-nitrosoglutathione (GSNO) have different properties from each other. In presence of oxygen, ac.$NaNO_2$ can generate nitrogen dioxide (NO_2), dinitrogen trioxide (N_2O_3), and nitric oxide (NO^+) [7]. The decomposition of ac.$NaNO_2$ is dependent on the acidity of the medium [8]. The formation of NO is also proportional with the formation of N_2O_3, a highly efficient nitrosating agent [7]. On the other hand, the decomposition of GSNO is dependent on light, thiols, metal etc [9]. GSNO can be decomposed through both homolytic and heterolytic fission. Homolytic fission of GSNO depends on the metals like Cu^{2+} but heterolytic decomposition is mainly predominated in the biological system. The effect of thiols on the decomposition of GSNO is also very complex. It has been reported that excess cysteine can contribute to increase the half-life of *S*-nitrosoglutathiones [10, 11]. On the other hand, thiols can increase the rate of the decomposition of *S*-nitrosoglutathiones. Thus, depending on the redox conditions, *S*-nitrosoglutathiones can be decomposed heterolytically and NO, NO^-, NO^+ reactive chemical species are formed [9]. NO derivatives, produced *in vivo*, can either be beneficial or deleterious to the organisms [1]. The toxicity of these compounds depends on the concentration of the dose along with the duration of the treatment. Choice of cell/organism also influences the effect of these compounds. These NO derivatives including peroxynitrite, *S*-nitrosothiols, nitrogen oxides etc. can influence the *in vivo* redox homeostasis, resulting in nitrosative stress [2, 3].

Thus, at the initial phase of the work, the effect of nitrosative stress agents upon the growth of *Saccharomyces cerevisiae* were evaluated under the specified experimental condition. *Saccharomyces cerevisiae* is a budding yeast and one of the best model to study the effect of nitrosative stress. Acidified sodium nitrite (inorganic) **[Fig. 6A]** and *S*-nitrosoglutathione (organic) **[Fig. 6B]** were chosen as the 'NO donor' in this study. This study was performed to determine the sub-toxic dose (the concentration of the respective agents where growth was almost similar to the control) of these two compounds on the growth of *Saccharomyces cerevisiae* strain Y190 (ATCC 96400).



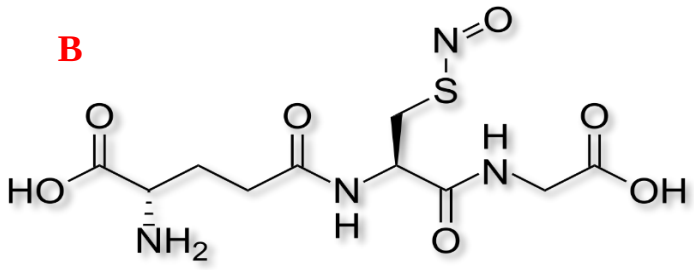


Fig. 6 Chemical formula of the reactive nitrogen species. **(A)** Sodium nitrite and **(B)** *S*-nitrosoglutathione (GSNO).

Results:

To observe the effect of ac. $NaNO_2$ and GSNO on the cell growth, *Saccharomyces cerevisiae* cells were grown in YPD medium and after 3 h, different concentrations of nitrosative stress agents were added and incubated overnight Under shaking condition. Following an overnight incubation, cell viability was determined. For the growth curve analysis, cell growth was monitored for atleast 12 h by measuring the optical density at 600 nm with one hour intervals.

It was observed that the cell viability of *Saccharomyces cerevisiae* cells were not altered in the presence of 0.5 mM ac.$NaNO_2$ as compared to the control (0 mM ac.$NaNO_2$). In presence of 1 mM and 3 mM ac.$NaNO_2$, under the same experimental conditions, cellular viability was significantly affected by nearly 25% and 50%, respectively **[Fig. 7A]**. Observed result indicated that 0.5 mM ac.$NaNO_2$ had no effect on the cell viability. Furthermore, specific growth rate was determined from growth curves. 0.5 mM ac.$NaNO_2$ treated cells showed no difference in specific growth rate (0.22 h^{-1}) as compared to the control **[Fig. 7B]**.

When a similar experiment was conducted with the treatment of various concentrations (0, 0.25, 0.5, 1 mM) of GSNO, cell viability of *Saccharomyces cerevisiae* cells were almost unaffected at 0.25 mM concentration of GSNO as compared to the control. Whereas, in presence of 0.5 and 1 mM GSNO, cell viability was significantly decreased by 30% and 60% respectively **[Fig. 8A]**. After that specific growth rates of control and 0.25 mM GSNO treated *Saccharomyces cerevisiae* cells were also determined in that condition. Observed result showed no significant difference in the specific growth rate (0.22 h^{-1}) of *S. cerevisiae* in presence of 0.25 mM GSNO as compared to the control **[Fig. 8B]**.

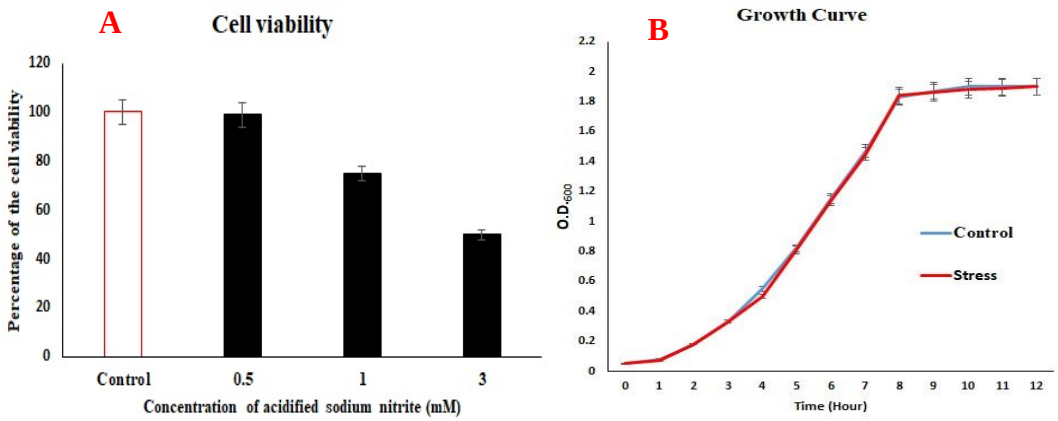


Fig. 7 Effect of acidified sodium nitrite on growth of *Saccharomyces cerevisiae* in YPD medium. (A) Cell viability assay of control (untreated) and treated (0.5 mM, 1 mM and 3 mM acidified sodium nitrite) *S. cerevisiae*. **(B)** Comparison of growth curves between control (untreated) and treated (0.5 mM) *S. cerevisiae*.

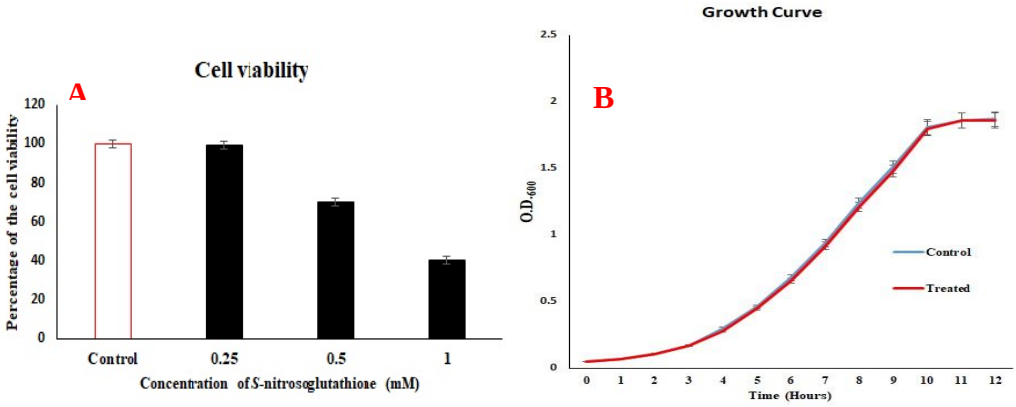


Fig. 8 Effect of *S*-nitrosoglutathione on growth of *Saccharomyces cerevisiae* in YPD medium. (A) Cell viability assay of control (untreated) and treated (0.25 mM, 0.5 mM and 1 mM acidified sodium nitrite) *S. cerevisiae*. **(B)** Comparison of growth curves between control (untreated) and treated (0.25 mM) *S. cerevisiae*.

Discussion:

The obtained results reveal some interesting insights regarding the effect of ac.$NaNO_2$ and GSNO, on the growth of *S. cerevisiae*. In the presence of higher concentrations of ac.$NaNO_2$ and GSNO, cell viability of *S. cerevisiae* was significantly decreased, indicating ac.$NaNO_2$ and GSNO has toxic effect on the cellular growth depending on their concentration. Acidified $NaNO_2$ and GSNO are well-known NO donor [4, 6, 7]. Thus, it can be assumed that nitrosative stress, generated by the action of ac.$NaNO_2$ and GSNO, was lethal for the cells. In addition to it, *S. cerevisiae* cells clearly showed more sensitivity to GSNO as compared to ac.$NaNO_2$.

Determination of the sub-toxic dose of these two agents was very important for all further experiments. The above mentioned experiments also gave insights for choosing the sub toxic doses of ac. $NaNO_2$ and GSNO. When the cells were treated with different concentration of ac.$NaNO_2$, It was found that the cell viability and specific growth rate was not altered in the presence of 0.5 mM ac.$NaNO_2$ as compared to the control whereas *S. cerevisiae* cells tolerated upto 0.25 mM GSNO and beyond this concentration the cell viability was drastically decreased. Thus, the sub-toxic doses were set to 0.5 mM and 0.25 mM for the treatment with ac.$NaNO_2$ and GSNO respectively.

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