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Research on the growth ability of *Saccharomyces cerevisiae* MN2 in the medium containing different concentrations of NaCl and NH_4^+ for applying in aquaculture

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ABSTRACT

Salinity is one of the factors affecting the growth and activity of microorganisms. The study of the salinity effect can indicate the range of microorganism applications. This study evaluated the effects of NaCl concentration on growth, amino acid and NH_4^+ absorption of yeast strain *Saccharomyces cerevisiae* MN2 to apply this strain in aquacultural environmental treatment. The results showed that the growth of strain MN2 was not affected by the NaCl concentration from 0–3%. The cell density reached 10^8 CFU/mL after 24 h of culture and was maintained for up to 48 h. The strain MN2 was able to absorb amino acids very well. The result was demonstrated by reducing amino acid content in the culture medium after 48 h, ranging from 1,700 mg/L (25,37%) to 2,500 mg/L (37,31%) compared with the control. In addition, the strain MN2 was also able to reduce the concentration of NH_4^+ in culture media, ranging from 1.53 mg/L (16.19%) to 2.4 mg/L (25.40%) compared with the control after 48 h. The ammonium in water affected aquaculture greatly. For strain MN2, ammonium concentration less than 75.4 mg/L did not affect growth. The results show that the yeast strain *S. cerevisiae* MN2 can grow well on the medium with high salt and ammonium concentration (3% and 75.4 mg/L, respectively). Therefore, it is possible to apply strain MN2 in aquaculture water treatment.

Keywords: NaCl concentration, NH_4^+ concentration, *Saccharomyces cerevisiae*, aquacultural treatment.

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INTRODUCTION

S. cerevisiae is a yeast species that can grow in environments with different salinity. Logothetis et al. showed that appropriate salt concentration can stimulate the osmosis of *S. cerevisiae* [1]. According to Logothetis et al., the culture of *S. cerevisiae* at high concentrations of NaCl can improve ethanol yield [2]. Some other authors also confirmed the salt tolerance of *S. cerevisiae* [3–6]. Besides, *S. cerevisiae* can also use ammonium and amino acids as nitrogen sources for growth. Magasanik (2003) reported ammonia assimilation by *S. cerevisiae* by two pathways: the reductive amination of 2-ketoglutarate, catalyzed by glutamate dehydrogenase, in which NADPH serves as the source of electrons, or by the ATP-dependent synthesis of glutamine from glutamate and ammonia catalyzed by glutamine synthetase [7]. According to Marini et al., (1997), *S. cerevisiae* can absorb ammonium at high concentrations (> 20 mM) [8]. *S. cerevisiae* can use amino acids as a nitrogen source, as reported by El-Samargy et al., [9]. Other authors' research also confirmed *S. cerevisiae* can use amino acids and ammonium as nitrogen sources [1, 6, 10, 11]. From the salt tolerance basis, using ammonium and amino acids as nitrogen sources of *S. cerevisiae* found that they can be applied in aquaculture water treatment.

In Vietnam, there has also much researche on the *S. cerevisiae*. However, most of them focus on researching the fermentation of *S. cerevisiae* [12, 13] and using biomass *S. cerevisiae* as an additional feed source for domestic animal. Phan Thi Hang et al., showed that the fermented feed using *S. cerevisiae* supported DM digestibility increasing by more than 5.4 %, protein increasing by more than 10.1% and the height of the villi in the duodenum increasing by more than 17% [14]. *S. cerevisiae* is also used in wastewater treatment. Research by Nguyen Thi Ha et al., supposed that *S. cerevisiae* can absorb heavy metals with a high efficiency of up to 95% for Pb^{2+} [15]. Therefore, the study of *S. cerevisiae* for treating aquaculture water helps to provide more information and apply this yeast.

Besides the benefits of aquaculture, it also brings some environmental problems. In aquaculture, leftovers, and animal waste harm livestock and pollute water sources, affecting environmental quality. The leftovers and waste in aquaculture contain much protein. In the process of decomposition these substances can produce a lot of NH_4^+, which is harmful to livestock. For shrimp culture water, according to the standards of QCVN 02-19:2014/BNNPTNT, the H_2S content must not exceed 0.05 mg/L, and the free NH_3 must not exceed 0.3 mg/L. Many products of microorganisms used to limit the formation of these harmful components. However, the application products of microorganisms for treatment are also facing some difficulties because of the difference in salinity between fresh, brackish water, and salt water.

The changing salinity significantly affects some microorganisms, so applying them to freshwater, brackish and marine water is difficult. This paper surveyed the growth of *S. cerevisiae* strain MN2 in different concentrations of salt and ammonium to orient to apply in aquaculture.

MATERIALS AND METHODS

Materials

S. cerevisiae MN2: from the collection of Department of Environmental Microbiology.

The yeast culture medium: Hansen media (g/L) including Yeast extract 1 g, pepton 5 g, $MgSO_4$ 3 g, KH_2PO_4 3 g, K_2HPO_4 3 g, agar 20 g.

Medium containing NH_4Cl(g/L) including $MgSO_4$ 3 g, KH_2PO_4 3 g, K_2HPO_4 3 g, NaCl 30 g, NH_4Cl 1–3 g.

Method

Cell density determination method

1 mL of the sample was added to 9 mL of sterilized saline to obtain a 10^{-1} dilution; then, 1 mL of the 10^{-1} suspension was added to 9 mL of saline to obtain a 10^{-2} dilution. Do the same

with subsequent dilutions until the required concentration is reached.

Three suitable dilutions, each replicated 3 times with 100 µL, were inoculated with Hansen medium and equally spread in a petri dish. Samples were then cultured in a 28°C incubator within 48 h. The samples were counted as the number of colonies on the plate. The density is determined by the following formula:

$$X = 10 \times ab \ \text{(CFU/mL)}$$

where: a- number of colonies; *b*- inversion of dilution concentration; *X*- cell density.

Method for determination of amino acids, ammonium and Salinity concentrations

The 150 mL of cultured solution of *S. cerevisiae* (at different NaCl concentrations) was centrifuged at 6,000 rpm and 4°C for 10 min to obtain the supernatant. The supernatant was used to analyze total amino acids, ammonium, and salinity. Total amino acids were determined by the method following TCVN 8764: 2012 [16]. Ammonium in solution was determined by the method following TCVN6179-1:1996 [17]. Salinity in the solution was determined by a Salinity meter (ATAGO - Japan).

Experimental set - up

Studying on the effect of salt concentration

The basic Hansen medium was prepared, then contributed into different flasks with the following NaCl concentrations: 0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0%; this medium was sterilized at 121°C for 15 min.

After the sterilization, the *S. cerevisiae* was inoculated to the media, cultured at 28°C, and shaken at 150 rpm. The sampling was taken at 0 h, 24 h, and 48 h to analyze the salinity, amino acid, ammonium concentrations and cell density.

Studying the effect of initial NH_4Cl at 3% salt concentration

The basic Hansen medium with 3% NaCl was prepared, then contributed into different flasks with the following NH_4Cl concentrations: 0.10, 0.15, 0.20, 0.25, and 0.30%; the medium was sterilized at 121°C for 15 min.

After the sterilization, the *Saccharomyces cerevisiae* was inoculated to the media at 28°C and shaken at 150 rpm. The sampling was taken at 0 h, 24 h, and 48 h to analyze the salinity, ammonium concentrations, biomass, and cell density.

RESULTS

Studying the effect of salt concentration on strain S. cerevisiae MN2

The experiment was carried out on Hansen medium with NaCl concentration ranging from 0–3.0%. The data of parameters: salinity, amino acid, and ammonium absorption and cell density in cultured media are summarized in Table 1.

The results indicated that all experiments' salinity concentrations decreased during culturing. In particular, the salinity concentration decreased sharply from 0 h to 24 h; this was also when the yeast grew strongly. From 24 h to 48 h, the salinity concentration was not significant. A question raised here is whether *S. cerevisiae* absorbs NaCl during growth or absorbs some minerals that can affect salinity. From an oceanographic view of salinity, it characterizes the minerality of seawater, which is understood as the total amount in grams of all dissolved solid minerals present in 1 kg of seawater. The salinity of the water is usually determined by the content of 11 main ions (Na^+, Ca^{2+}, Mg^{2+}, Fe^{3+}, NH_4^+, Cl^-, SO_4^{2-}, HCO_3^-, CO_3^{2-}, NO_2^-, NO_3^-) because they account for 99.99% of the total amount of minerals dissolved in water. In the Hansen medium, there are also minerals: Mg^{2+}, and K^+, so the salinity of the initial medium is usually higher than the amount of NaCl added to the medium. However, during the growth of the MN2 strain, the salinity in both the medium supplemented with and without NaCl decreased, indicating that the reduced salinity could be due to the yeast absorbing minerals in the medium rather than NaCl.

Table1. Effect of salt concentrations on growth, amino acid absorption and limited formation of ammonium of *S. cerevisiae* MN2

NaCl concentration (%)	Saltlity (%)			Cell count (CFU/mL)			Total amino acid (mg/L)			Total amoni (mg/L)		
	0 h	24 h	48 h	0 h	24 h	48 h	0 h	24 h	48 h	0 h	24 h	48 h
Control (0%)	4.8	4.8	4.8	-	-	-	6,700	6,700	6,700	9.45	9.45	9.45
0	4.8	2.0	2.0	2.2×10^5	3.0×10^8	1.8×10^8	6,700	6,400	5,000	9.45	8.55	7.75
0.5	5.2	2.4	2.3	2.2×10^5	7.0×10^8	1.3×10^8	6,700	6,400	5,000	9.45	8.40	7.69
1.0	5.6	3.0	2.8	2.1×10^5	7.6×10^8	1.5×10^8	6,700	6,400	5,000	9.45	8.05	7.42
1.5	6.0	3.5	3.5	2.3×10^5	6.4×10^8	2.5×10^8	6,700	6,500	4,800	9.45	8.80	7.84
2.0	6.5	4.0	3.9	1.9×10^5	5.2×10^8	1.3×10^8	6,700	6,500	4,700	9.45	8.83	7.92
2.5	6.7	4.2	4.0	2.3×10^5	5.4×10^8	1.4×10^8	6,700	6,200	4,200	9.45	8.85	7.67
3.0	7.1	4.7	4.6	1.8×10^5	3.9×10^8	2.6×10^8	6,700	6,500	4,200	9.45	8.00	7.05

The growth of *S. cerevisiae* MN2 was affected when NaCl was added into the medium with a concentration from 0 to 3%. The CFU index in all different media reached 10^8 CFU/mL after 24 h of culturing, and after 48 h, the cell density remained around 10^8 CFU/mL. The results indicated that the NaCl addition did not affect the growth of the strain MN2. Similar research was also reported by Stilianos et al., (2010). The authors reported that yeast could grow when the NaCl concentration was up to 3% with a cell density of 10^8 CFU/mL [18]. The amino acids in the culture medium decreased gradually when the NaCl concentration ranged from 0 to 3%. The amount of amino acids after 24 h has only decreased by less than 10%. Meanwhile, after 48 h, the amino acid reduction was highest, reaching 37.31% at the initial NaCl concentration of 2.5 and 3%; the lowest reduction is 25.31% at the initial concentration of NaCl 0–1% (with initial amino acid content in the culture medium is 6,700 mg/L) (Fig. 1). Besides, the ammonium content in the culture medium also changed. The ammonium content decreased gradually in all media with different concentrations of NaCl. The sharpest reduction rate was in the medium-added NaCl at 3%, reaching 25.4% (Fig. 2). The results of amino acid and ammonium absorption are inconsistent with previous studies of Takagi et al., (2000) and Magasnik et al., (2001), which reported that the *S. cerevisiae* was capable of using nitrogen as amino acids and ammonium as nitrogen sources for their growth [6, 7].

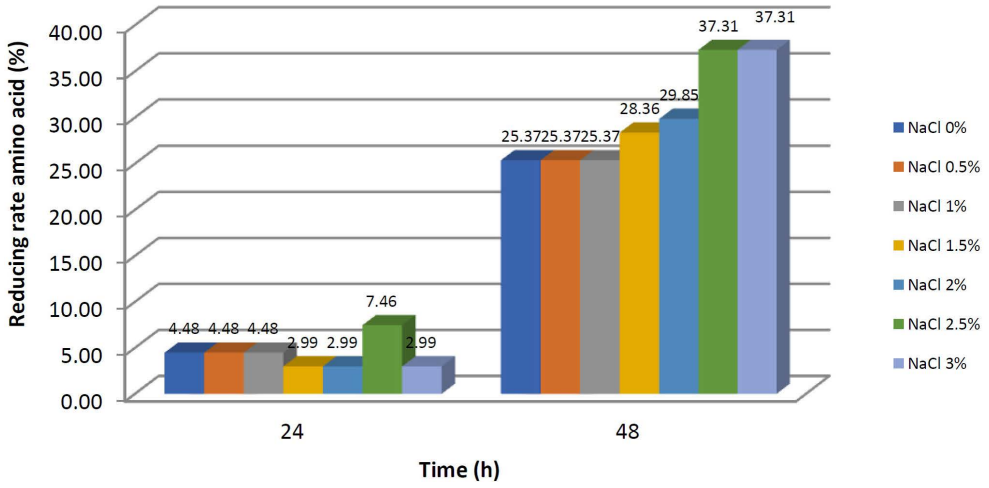

Figure 1. Effect of NaCl on amino acid absorption capacity of MN2 strain

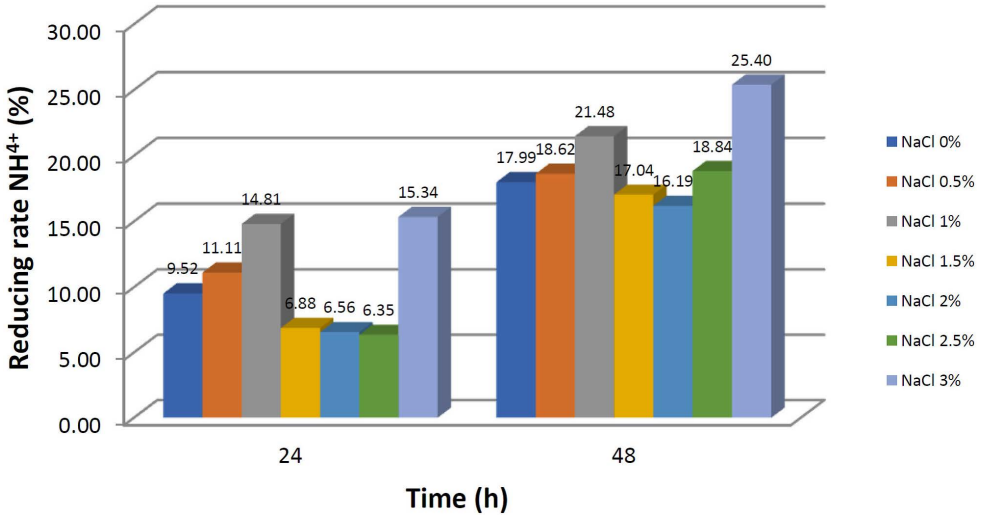

Figure 2. Effect of NaCl on ammonium absorption capacity of MN2 strain

The above results showed that the yeast strain MN2 could grow sufficiently with the NaCl concentrations 0–3%. When the salt concentration increases, the absorption of amino acids and ammonium of MN2 strain also increases, which is meaningful and promising information for practical applications. The result is inconsistent with the study of Stilianos et al., (2010) [6]. However, in that study, the authors also mentioned ethanol formation and the ability to consume sugar but did not mention the absorption ability of amino acids and ammonium. So, it is a different point of this study.

According to some studies, the ammonium source is not the preferred nitrogen source for *S. cerevisiae* [6–8, 11, 19, 20], and excess ammonium in water sometimes causes a negative effect, especially with aquaculture. Therefore, the effect of the ammonium concentration on the growth of this strain should be studied further in the following section.

Studying the effect of NH_4Cl concentration on the growth of MN2 strain at 3% NaCl concentration

Ammonium is the nitrogen source for *S. cerevisiae* to grow, but it is also a contaminant of the aquaculture environment. The results of the salinity effect mentioned above showed that the ammonium absorption capacity of strain MN2 was relatively high, but the highest initial ammonium content was only 9.7 mg/L. So if the amount of ammonium in the environment increases, how does the ammonium absorption of strain MN2 changes? An experiment was conducted with a mineral medium supplemented with NH_4Cl salt at 0.1–0.3% and NaCl at 3%. The results are summarized in Table 2.

With NH_4Cl at 0.1–0.3%, the ammonium in the initial culture was relatively high, ranging from 37.3 mg/L to 75.4 mg/L. The results indicated that the salt concentration in the culture medium of the MN2 strain was not significantly different if NaCl (3%) and NH_4Cl (0.1% to 0.3%) were added into the medium.

The difference between the strain grown in medium with NH_4Cl source compared to basic Hansen was observed, in which the log phase came later in the medium with NH_4Cl (after 48 h compared with 24 h of basic Hansen). The total biomass at 48 h demonstrated that if the concentration of NH_4Cl in the medium increased, the total biomass tended to decrease. The highest biomass total reached 5.95 g/L at 0.1% of NH_4Cl, and the lowest was 4.9 g/L at 0.3%. However, the reduction in biomass was not significant, so it can be confirmed that NH_4Cl content from 0.1% to 0.3% did not affect the growth of the MN2 strain (Fig. 3).

Table 2. Effect of NH_4Cl concentration and ammonium concentration on growth of Saccharomyces cerevisiae MN2 medium

NH_4Cl concentration	Saltlity (%)			Cell count (CFU/mL)			Biomass after 48 h (g/L)	Total amoni (mg/L)		
	0 h	24 h	48 h	0 h	24 h	48 h		0 h	24 h	48 h
0.10%	6.4	4.0	3.8	1.6×10^5	9.7×10^7	6.7×10^8	5.96	37.3	17.7	1.2
0.15%	6.3	3.9	3.7	1.6×10^5	9.0×10^7	6.8×10^8	5.95	52.4	35.2	15.0
0.20%	6.3	3.9	3.8	1.6×10^5	9.9×10^7	6.4×10^8	5.94	61.3	46.2	21.5
0.25%	6.3	3.8	3.8	1.6×10^5	3.2×10^7	5.9×10^8	4.92	66.6	52.4	28.3
0.30%	6.4	4.0	3.8	1.6×10^5	2.4×10^7	5.7×10^8	4.90	75.4	64.6	40.0

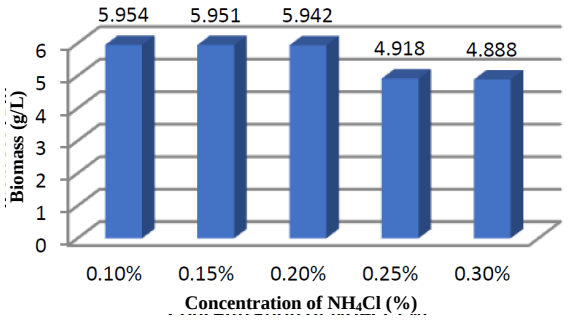

Figure 3. Effect of NH_4Cl on growth of S. cerevisiae MN2

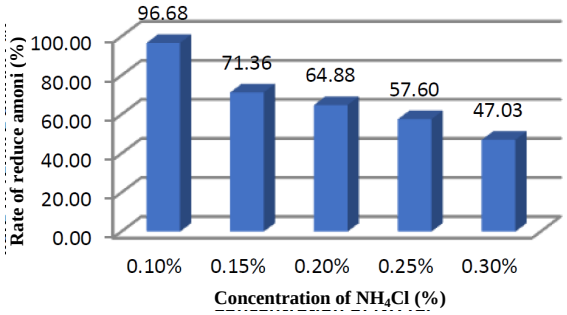

Figure 4. Effect of NH_4Cl on ammonium absorption capacity of MN2

In addition to the salinity and growth ability, the assessment of NH_4Cl depletion is also essential. How will increasing the ammonium content in the culture medium affect absorption capacity? The results indicated that when the concentration of NH_4Cl in the medium changed from 0.1–0.3%, the strain MN2 had an excellent absorption capacity. The highest absorption was up to 96.68% at 0.1% ammonium concentration and gradually decreased as the concentration of NH_4Cl increased, but the lowest absorption rate still reached 47.03% (Fig. 4). The above results demonstrated that *S. cerevisiae* MN2 could grow and absorb ammonium even when the ammonium in the medium, reached 75,4 mg/L.

According to research by some authors, high ammonium concentration in the aquaculture environment affects livestock. Rostami et al., (2019) reported ammonium concentration in the 13–19 mg/L medium. The growth of shrimp was lower than the medium with a lower concentration. At 19 mg/l ammonium concentration, the death rate of shrimp can reach 80.55% [11]. According to Robinette (1976) and Meade (1985), the toxic levels of unionized ammonia for short-term exposure are usually at 0.6 and 2.0 mg/L for pond fish, and sub-lethal effects may occur at 0.1 mg/L to 0.3 mg/L [2, 18]. Therefore, reducing ammonium in aquaculture water is vital for livestock productivity and quality. The strain MN2 has ammonium absorption well, which is promising in practical applications.

The above results confirmed that the NaCl concentration in the medium from 0 to 3% did not affect the growth of strain MN2. This study provided the basic data for applications of *S. cerevisiae* MN2 in aquaculture water treatment. This yeast strain could use amino acids and ammonium as a nitrogen source for their growth.

CONCLUSION

The concentration of NaCl 0–3% did not affect the growth, amino acid absorption, and ammonium absorption of the strain *S. cerevisiae* MN2. The cell density reached 10^8 CFU/mL after 24 h; the highest amino acid and ammonium absorption was 2,500 mg/L (37.31%) and 2.4 mg/L (25.4%) after 48 h. The

strain MN2 could use NH_4Cl as a nitrogen source for the growth. 75.4 mg/L of ammonium concentration in the medium did not affect the growth of *S. cerevisiae* MN2, cell density still reached 10^8 CFU/mL, and ammonium absorption was 35.4 mg/L, equivalent to 47.03% after 48 h.

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