

**EVALUATION OF THE GROWTH OF *Vibrio natriegens* STRAINS
IN A MEDIUM CONTAINING CHITIN DERIVATIVES
AND SHRIMP SHELL HYDROLYSATE**

**Le Tuan, Nguyen Thanh Hung, Nguyen Tuan Viet,
Vu Thi Kieu Oanh, Pham Tuan Anh, Le Thanh Ha***

School of Chemistry and Life Sciences, Hanoi University of Science and Technology,
Ha Noi, Vietnam

Received 31 August 2023; accepted 6 December 2023

ABSTRACT

Chitin from crustacean waste can be the future substrate for bioindustry towards the circular economy concept, especially for countries having large production of sea products like Vietnam. However, the chemical conversion of chitin into monomers implies high amounts of NaCl in a mixture with glucosamine, N-acetyl glucosamine, and acetate. Thus, a bacterial strain that can tolerate high salt concentration, and use chitin monomers as the sole carbon source such as *Vibrio natriegens* holds great potential in producing bioproducts from chitin derivatives. In this study, *V. natriegens* strains 10.3 and N5.3 isolated from Vietnam were compared with the reference strain *V. natriegens* DSM 759 for their growth performances in medium containing separately glucose and chitin monomers. Strain N5.3 showed the best growth rate among the 3 tested strains, exceptionally in medium containing glucosamine with nearly 1.5 times faster than strains 10.3 and DSM 759. Strain N5.3 cultured in bioreactor at 30 g/L NaCl indicated growth rate ranging from 0.614 to 0.881 h⁻¹ in medium containing glucose, glucosamine, N-acetyl glucosamine, and shrimp shell hydrolysate. The formation of acetate was observed during the exponential growth of strain N5.3 in medium with glucose and N-acetyl glucosamine but not in medium with glucosamine or shrimp shell hydrolysate. The faster growth of all tested strains on N-acetyl glucosamine compared to glucosamine suggested metabolism of these substrates of *V. natriegens* similar to *Escherichia coli*.

Keywords: *Vibrio natriegens*, chitin derivatives, glucosamine, N-acetyl glucosamine, shrimp shell hydrolysate, cell growth.

Citation: Le Tuan, Nguyen Thanh Hung, Nguyen Tuan Viet, Vu Thi Kieu Oanh, Pham Tuan Anh, Le Thanh Ha, 2023. Evaluation of the growth of *Vibrio natriegens* strains in a medium containing chitin derivatives and shrimp shell hydrolysate. *Academia Journal of Biology*, 45(4): 73–82. <https://doi.org/10.15625/2615-9023/18777>

*Corresponding author email: ha.lethanh@hust.edu.vn

INTRODUCTION

Chitin is the second most abundant polysaccharide in nature after cellulose (Kostag & El Seoud, 2021). In nature, chitin is the main composition of crustacean shells, insect exoskeleton, plant, and fungal cell walls (Arnold et al., 2020; Hou et al., 2020). According to VASEP, the production of shrimp in Vietnam reached 1.08 million tons in 2022, with a growth of 8.5% compared to 2021. Shrimp waste, mainly head and body shell, accounted for 45–60% of shrimp weight and contains 15–17% w/w of chitin. It is estimated that more than 50,000 tons of chitin can be recovered from that amount of shrimp shell waste. Products obtained from the hydrolysis of chitin, i.e., D-glucosamine (GlcN) and N-acetyl D-glucosamine (GlcNAc), can be used as nutrition sources for the culture of several microorganisms, in particular *Vibrio natriegens* (Hoffart et al., 2017; Long et al., 2017). Thus, the valorization of shrimp processing waste and obtaining chitin from this waste represents a valuable potential for a double target: reducing environmental problems and creating new feedstock for the bio-industry.

V. natriegens has been the fastest growing non-pathogenic bacteria known so far, with a generation time of less than 10 min on BHI medium (Eagon, 1962; Lee et al., 2019). Relating to its natural living environment, *V. natriegens* requires sodium ions for growth. According to Hoffart et al. (2017) and Thiele et al. (2021), *V. natriegens* can grow in a medium containing 6 to 30 g/L NaCl but it cannot grow without or with 58.44 g/L NaCl. Consequently, most of the published studies on *V. natriegens* were performed under an optimal salt concentration of 15 g/L NaCl. In the minimal medium containing glucose as the sole carbon source, *V. natriegens* aerobically exhibited a growth rate of $\mu = 1.48 - 1.7 \text{ h}^{-1}$. Besides, it can metabolize more than 60 different types of carbon sources, including chitin derivatives (Eagon, 1962; Hoffart et al., 2017; Erian et al., 2020). It was reported that *V. natriegens* showed a significant higher growth rate in the medium containing GlcNAc

($\mu = 1.74 \text{ h}^{-1}$) than medium containing GlcN ($\mu = 0.68 \text{ h}^{-1}$) (Hoffart et al., 2017). Recent publications proved *V. natriegens* as the prominent workhorse for the production of several bio-products such as L-alanine, L-lysine, L-arginine, L-histidine, propane-diol, butane-diol, antimicrobial peptide and beta-carotene (Ellis et al., 2019; Erian et al., 2020; Stella et al., 2021; Zhang et al., 2021; Schwarz et al., 2022).

Chitin can be converted into monomers by chemical or biochemical (enzyme) methods. However, the application of the enzymatic method on the industrial scale is hindered by high cost, so the acid hydrolysis method is widely used in conventional processes to produce chitin monomers and oligomers. To date, HCl is used to degrade the chitin efficiently to yield GlcN mono HCl as a principal product. When this product is used as the substrate for submerged cell cultivation, the neutralization of HCl by NaOH generally results in high salt concentration (NaCl) in the medium, which represents an inhibitor effect on most bacteria. In this context, *V. natriegens* appears as the suitable candidate, which can metabolize chitin monomers at high salt concentrations.

In this study, we evaluated and compared the growth of *V. natriegens* DSM 759 (reference strain) and the two strains isolated from seawater in Nam Dinh, Vietnam (strains N5.3 and 10.3) in medium containing chitin monomers at high saline concentration (30 g/L NaCl). The fastest growing strain will be further investigated at a bioreactor scale using shrimp shell hydrolysate (SSH) as the main substrate.

MATERIALS AND METHODS

Microorganisms

The two strains *V. natriegens* N5.3 and *V. natriegens* 10.3 were isolated from seawater in Nam Dinh, Vietnam. Their identity was determined based on their morphology and 16S rRNA gene sequences which were submitted in GenBank with the accession number ON003474 and ON003471, respectively.

The reference strain *V. natriegens* DSM 759 (ATCC 14078, NCMB 857) (Payne, 1958) was obtained from the German Collection of Microorganisms and Cell Cultures.

Media composition

Media compositions included VN minimal salt solution (composed per liter: 30 g NaCl, 1 g K₂HPO₄, 1 g KH₂PO₄, 0.5 g (NH₄)₂SO₄, 0.05 g yeast extract, 0.25 g MgSO₄·7H₂O supplemented with 5 g/L CaCO₃, 1% oligo trace elements 100X stock solution (composition per liter as follows: 5 g EthyleneDiamineTetraacetic Acid, 1.64 g FeSO₄·7H₂O, 1.00 g MnSO₄·H₂O, 0.03 g CuSO₄·5H₂O, 0.10 g ZnSO₄·7H₂O, and 2 mg NiSO₄·6H₂O) (Hoffart et al., 2017), and 10 g/L (flask scale) or 20 g/L (bioreactor scale) of one carbon source Glc, GlcN, GlcNAc or SSH. Prior to cell culture, the medium was supplemented with 1 mg/L biotin and 1 mg/L thiamin (filter sterilized).

Chemical reagents

GlcN.HCl and GlcNAc (> 99% purity) were purchased from Suntze Chemical Co., Ltd., Vietnam. Other reagents were purchased from Samchun Pure Chemical Co., Ltd., Korea, or Sigma Aldrich.

Analyses of chitin monomers, glucose, and acetate

The concentration of GlcN and Glc were analyzed by using the DNS method (Miller, 1959). The concentration of GlcNAc and acetate were analyzed by HPLC using an Aminex HPX-87H column at 60°C, mobile phase 10 mM H₂SO₄, flow rate 0.6 mL·min⁻¹ (Li et al., 2023). UV detector at $\lambda = 210$ nm. For both DNS and HPLC analyses, all standard concentrations vary from 0.1 to 1.0 g/L.

Growth parameters

Samples taken during cell culture were treated with 6M HCl (ratio 1:10) to dissolve inorganic precipitates (mainly CaCO₃). Cell growth was evaluated by optical density at $\lambda = 600$ nm (APEL PD-3000UVE

spectrophotometer). For determining cell dry weight (CDW), 2 mL (for flask cultivation) or 10 mL (for bioreactor cultivation) of culture suspension was centrifuged at 10,000 rpm, for 10 min at 10 °C. The cell biomass was washed twice with 0.9% NaCl solution and was then dried at 85 °C until constant weight. The growth rate, μ (h⁻¹), the biomass yield $Y_{X/S}$ (g/g) and the biomass-specific substrate consumption rate q_s (g/g.h) were calculated following Hoffart et al. (2017).

Preparation of shrimp shell hydrolysate

Chitin from shrimp shell was firstly ground to particles less than 0.1 mm in size and then hydrolyzed by concentrated HCl according to the method described by Einbu et al. (2007) with some modification (HCl concentration equal to 9 M, solid/liquid ratio of 1/2, temperature 80 °C, duration 4 hours). The liquid phase obtained after acid hydrolysis was then filtered to remove impurities with subsequent detoxification by activated charcoal. Finally, excess HCl in the hydrolysate was neutralized by NaOH until pH ~ 7.0. The concentrations of reducing sugar and NaCl in shrimp shell hydrolysate were 94.3 g/L and 165.4 g/L, respectively. HPLC analysis of SSH indicated 0.7 g/L of GlcNAc and 20.56 g/L of acetate. To prepare the medium containing shrimp shell hydrolysate, 1 volume of SSH was diluted with 4.51 volume of distilled water to reach the final concentration of NaCl equal to 30 g/L. Then commercial GlcN was added to ensure a final concentration of reducing sugar equal to 20 g/L.

Strain inoculum

One colony of *V. natriegens* from the Luria Bertani (LB) agar plate was activated overnight in Luria Bertani broth medium (composed per liter of 10 g/L tryptone, 5 g/L yeast extract, and 30 g/L NaCl) at 30 °C on a rotary shaker at 150 rpm. The cell pellet was obtained by centrifuging at 20 °C; 6,000 rpm, re-suspended in 0.9% NaCl and served as the seed culture.

Cultivation of *Vibrio natriegens*

Small scale cultivation (culture volume = 50 mL) was performed in 250 mL baffled flask at 30 °C, shaking at 150 rpm for 24 h. pH was maintained by adding 3-(Nmorpholino) propanesulfonic acid (MOPS) to a final concentration of 10 g/L.

Bioreactor scale cultivation (1,000 mL) was carried out using Biostat B plus controller paired with a 2 L UniVessel glass bioreactor (Sartorius). pH was automatically regulated by using 4M NaOH and 4M HCl. Other culture conditions: temperature 30 °C, airflow rate 1–4 vol/vol/min and mixing rate 200–1,500 rpm were automatically adjusted to maintain pO₂ values above 20%.

For both scales, the initial OD of 600 nm was 0.1. Samples were taken during cultivation progress for analyses of OD₆₀₀, CDW, reducing sugar and HPLC.

Data analysis

Data analysis was performed using Microsoft Excel®.

Abbreviations

Glc: Glucose; GlcN: D-glucosamine; GlcNAc: N-acetyl-D-glucosamine; SSH: Shrimp shell hydrolysate.

RESULTS AND DISCUSSION

Growth of *Vibrio natriegens* strains in mediums containing chitin derivatives at flask scale

For shaking flask cultivation, pH values during cultivation were effectively maintained using the combination of MOPS and CaCO₃ (data now shown). In Figure 1, all strains were able to effectively metabolite chitin derivatives (GlcN and GlcNAc) equivalent to glucose (Glc).

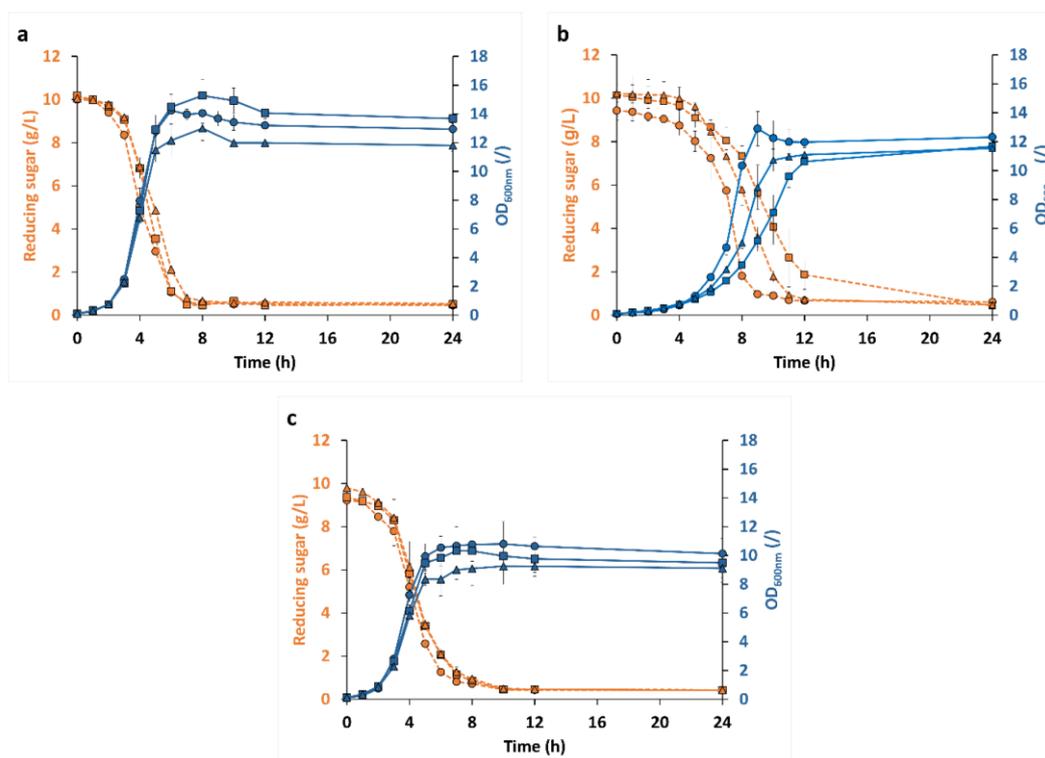


Figure 1. Growth of *Vibrio natriegens* strains N5.3 (●), 10.3 (■), and DSM 759 (▲) on medium containing Glc (a), GlcN (b), GlcNAc (c). The continuous line indicated value of OD at 600 nm, dashed line indicated value of reducing sugar during cultivation. Triplicate experiments were performed, and error bars represent standard deviation

On a minimal medium with glucose as the sole carbon source (Fig. 1a), it was observed that cell growth and substrate consumption of all three strains exhibited almost similar trends. Maximum growth rates of strains N5.3, 10.3 and DSM 759 in medium containing glucose (Table 1) were lower than the typical μ_{max} which ranged between 1.5 h⁻¹ and 1.7 h⁻¹ in shaking flask cultivation (Hoffart et al., 2017; Long et al., 2017; Ellis et al., 2019; Thoma & Blombach, 2021; Thoma et al., 2022). This can be explained by the cultivating temperature (T = 30 °C) in this

study was lower than that in all previous studies (T = 37 °C). In addition, it was reported that the growth rate of *V. natriegens* DSM 759 surged from 0.79 h⁻¹ at 30 °C to 1.36 h⁻¹ at 37 °C (increasing 1.7 times) according to Thiele et al. (2021).

At the end of the exponential phase, strain 10.3 reached slightly higher cell density than N5.3 and DSM 759 by optical measurement. This was confirmed by the cell dry weight analyses, that indicated the highest CDW value belonging to strain 10.3, and the lowest belonging to strain DSM 759 (Table 1).

Table 1. Growth parameter of *Vibrio natriegens* strains N5.3, 10.3, and DSM 759 on Glc, GlcN, and GlcNAc

Substrate	Strain	μ_{max} (h ⁻¹)	CDW (g.L ⁻¹)
Glc	N5.3	1.130 ± 0.010	3.611 ± 0.117
	10.3	1.043 ± 0.004	3.822 ± 0.078
	DSM 759	1.036 ± 0.041	3.469 ± 0.240
GlcN	N5.3	0.625 ± 0.013	4.174 ± 0.176
	10.3	0.405 ± 0.010	4.398 ± 0.057
	DSM 759	0.474 ± 0.009	4.256 ± 0.362
GlcNAc	N5.3	1.117 ± 0.036	2.094 ± 0.132
	10.3	0.999 ± 0.042	2.252 ± 0.091
	DSM 759	0.968 ± 0.072	2.141 ± 0.256

In a minimal medium using GlcN as the sole carbon source, the growth of three tested strains showed significant differences (Fig. 1b). Strain N5.3 exhibited the highest growth rate, which was 1.3 times faster than the growth rate of the reference strain DSM 759. In a minimal medium supplemented with GlcNAc, all strains showed small differences in growth rates and substrate consumption rates (Fig. 1c). It was interesting that *V. natriegens* grew quicker in a medium containing GlcNAc than a medium containing GlcN, with a factor of 1.8 to 2.5 in μ_{max} depending on strains. The metabolism of GlcN and GlcNAc in *Escherichia coli* requires GlcN6P isomerase (*nagB*) for the conversion of GlcN6P into Fructose6P. Similar substrate uptake behaviour was reported for *E. coli* and was explained by the allosteric activation of *nagB* by GlcNAc6P (Peri et al., 1990; Alvarez-Añorve et al., 2005). However, the previous studies on the growth of *V. natriegens* in

medium containing chitin monomers were still limited. When *V. natriegens* DSM 759 was cultured in the minimal salt medium at 37 °C, Hoffart et al. (2017) observed its growth rate reached 0.68 h⁻¹ and 1.74 h⁻¹ in medium containing GlcN and GlcNAc, respectively. Using the same strain DSM 759, Ellis et al. (2019) reported a superior value of μ_{max} (reaching 1.5 h⁻¹) in an M9 medium supplemented with 4 g/L GlcNAc. Considering the differences in cultivation temperature, the results of this study were in the same magnitude as the previous studies. Together, it was suggested that *V. natriegens* possessed metabolic pathways of GlcN and GlcNAc similar to *E. coli*.

It is important to note that, the growth of strain N5.3 in the presence of Glc exhibited an abnormal decrease of cell density right after reached its peak at t = 9 h (Fig. 1b). Similar

phenomenon was also observed by Thiele et al. (2021) for strain DMS 759 and was attributed to spontaneous activation of prophage regions Δ VNP1 and Δ VNP2, leading to the death of cell suddenly (Wiegand et al., 2018; Pfeifer et

al., 2019). This observation suggested that the prophage region may also exist in *V. natriegens* N5.3. Further genetic investigations must be performed to allow an insight into this hypothesis.

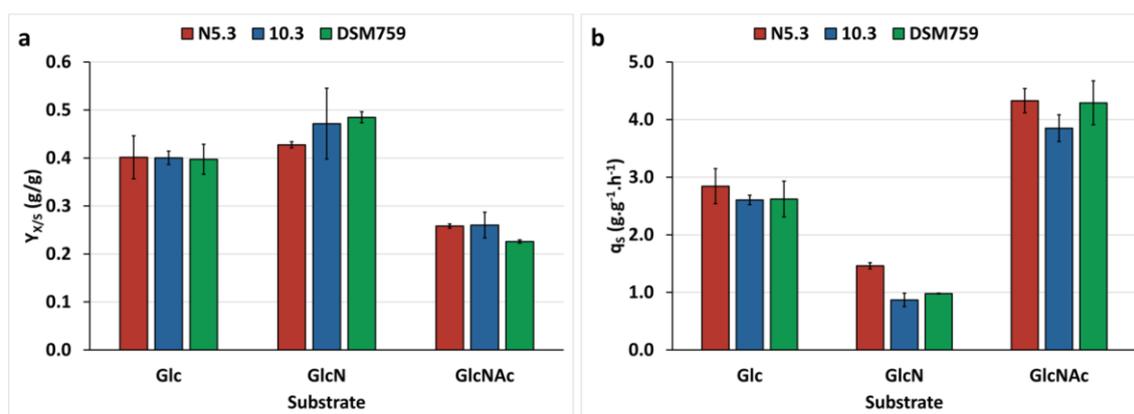


Figure 2. Substrate-specific biomass yield (a) and substrate uptake rate (b) of *Vibrio natriegens* strains at 30 g/L NaCl

Figure 2a illustrates substrate-specific biomass yield of three investigated strains on Glc and chitin derivatives. Similar values of $Y_{X/S}$ of approximately 0.4 $\text{g}_{\text{CDW}}/\text{g}_{\text{Glc}}$ were obtained for all strains. This result showed good agreement with previous studies, reported $Y_{X/S} = 0.36\text{--}0.44 \text{ g}_{\text{CDW}}/\text{g}_{\text{Glc}}$ (Hoffart et al., 2017; Long et al., 2017; Erian et al., 2020; Thoma & Blombach, 2021). It was interesting that an enhancement of up to 20% in biomass yield was observed on GlcN for all strains while a reduction was recognized on GlcNAc. On Glc and under fully aerobic conditions, it was reported that at least 80% of the substrate was metabolized through Embden-Meyerhof-Parnas pathway while the rest was directed to the Pentose Phosphate pathway (Eagon & Wang, 1962; Long et al., 2017). This resulted in a 50% C mol basis of Glc was converted to biomass and the rest was equally redirected to acetate and CO_2 (Long et al., 2017). As substrates were efficiently assimilated during exponential growth (Figs. 1b, c), it seems that the carbon flux entering *V. natriegens* cell was profoundly driven to biomass in case of presenting GlcN but preferable to secondary product formation and/or CO_2 for GlcNAc.

Regarding the substrate uptake rate q_s (Fig. 2b), the obtained values on Glc for three strains were slightly lower than the reported value from the previous studies, which varied between 3.5 and 3.9 $\text{g}_{\text{Glc}} \cdot \text{g}_{\text{CDW}}^{-1} \cdot \text{h}^{-1}$ (Hoffart et al., 2017; Long et al., 2017). This can be explained by the decrease in the growth rate at a lower temperature (30 °C) compared to the higher temperature (37 °C) leading to a lesser substrate uptake rate.

With the aims of seeking the most adapted *V. natriegens* strain for biovalorization of chitin waste, a fast growth rate on both GlcN and GlcNAc are required because these are the main monomers of chitin. However, as GlcN was generally reported as the main component of chitin hydrolysate, the uptake and growth rates on GlcN was considered as the critical criteria for selecting strain. In this specific context, it was suggested that strain N5.3 is the best candidate to become the micro-factory producing molecules of interest from chitin derivatives because of its highest growth rate on both GlcN and GlcNAc.

Growth of *Vibrio natriegens* N5.3 in mediums containing chitin derivatives and shrimp shell hydrolysate at bioreactor scale

The growth of *V. natriegens* N5.3 was evaluated at a bioreactor scale with different substrates (Figs. 3a, 3b). The cultivation was successfully performed under aerobic conditions (Fig. 3c) and pH values were maintained at 7.5 ± 0.2 (data not shown). Under these conditions, strain N5.3 grew faster on GlcNAc than SSH, Glc, and GlcN (Fig. 3a and Table 2). The decrease in reducing sugar during cultivation (Fig. 3b) was in good

agreement with cell growth (Fig. 3a) and oxygen consumption (Fig. 3c). Compared to flask scale cultivation (Table 1), the lower growth rate of *V. natriegens* N5.3 in bioreactor cultures may be attributed to the change of initial substrate concentration from 10 g/L (flask scale) to 20 g/L (bioreactor scale). For *V. natriegens* strain DSM 759, Peng et al. (2020) also obtained a modest μ_{max} of 0.87 h^{-1} on VN minimal medium supplemented by 20 g/L glucose, while a value of $1.5 - 1.7 \text{ h}^{-1}$ were reported for this strain on the same medium with 10 g/L glucose (Hoffart et al., 2017; Long et al., 2017).

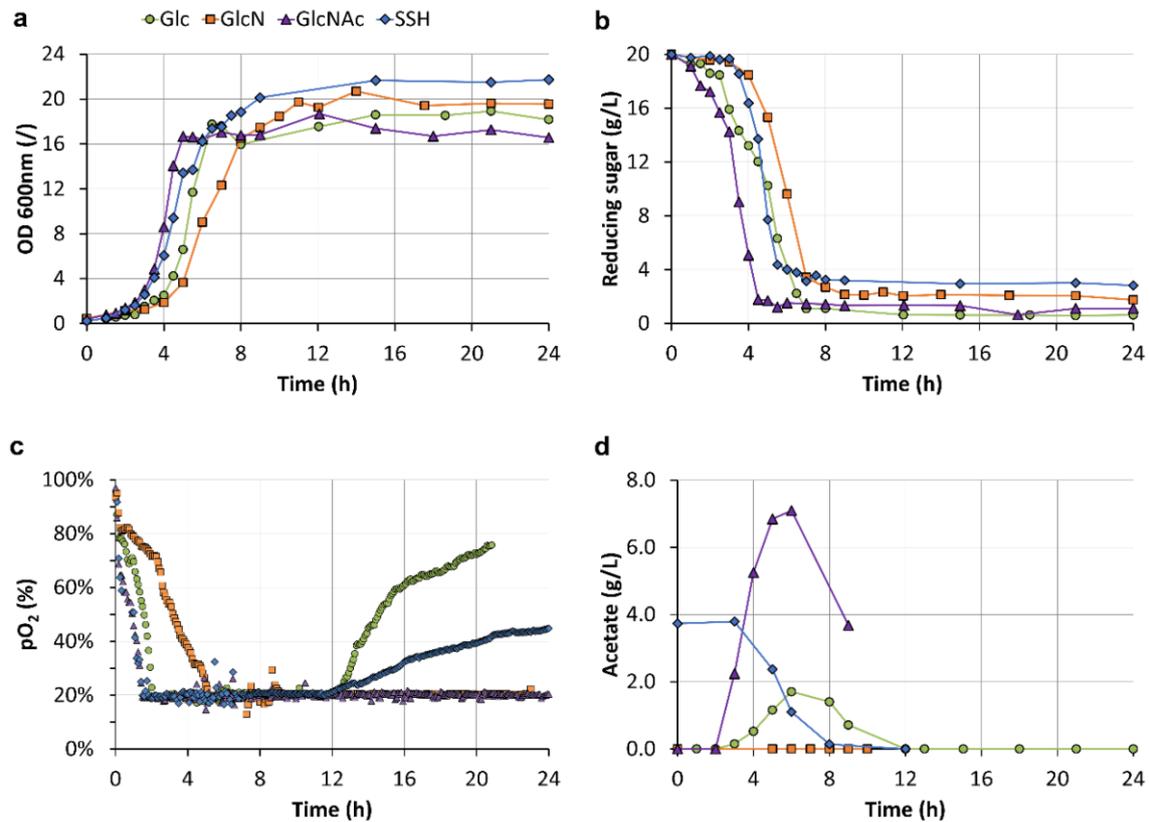


Figure 3. Growth of *Vibrio natriegens* N5.3 on minimal medium supplemented by different substrates at bioreactor scale, 30 g/L NaCl

It is interesting that there was no accumulation of acetate during the growth process of strain N5.3 on GlcN however opposite trends were observed in the medium containing GlcNAc or Glc (Fig. 3d). Interestingly for the culture using SSH as a

carbon sources, acetate was consumed during the exponential growth (Fig. 3d). It was reported that *V. natriegens* produced acetate from glucose under aerobic conditions, and several organic acids could be accumulated due to the lack of oxygen or under anaerobic

conditions (Hoffart et al., 2017; Thiele et al., 2021). It was also estimated that 25% of the carbon flux from glucose entering *V. natriegens* cell is redirected to form acetate and only 50% resulted in biomass (Long et al., 2017). These statements were in good agreement with our results on Glc, indicating an increase in acetate concentration up to 1.9 g/L during exponential growth. On GlcNAc, the surge in acetate concentration during exponential growth can be firstly explained by its formation as the by-product (Long et al., 2017). Besides, the metabolism of GlcNAc in *E. coli* includes the transportation of GlcNAc across the cell membrane, followed by phosphorylation by *NagE* encoded transporter. Then acetate was released when GlcNAc-6-P was converted to GlcN-6-P by deacetylase (Peri et al., 1990). As acetate can be also a carbon source for *V. natriegens* (Hoffart et al., 2017), strain N5.3 started to use acetate since the depletion of Glc or GlcNAc (Fig. 3d from $t = 6$ h).

For the cultivation of *V. natriegens* N5.3 in a medium containing shrimp shell

hydrolysate (SSH), it was observed that no acetate was produced, which was similar to when this strain grew on GlcN (Fig. 3d). The decrease in concentration of both acetate (Fig. 3d) and total reducing sugar (Fig. 3b) during exponential growth may suggest a co-consumption of both acetate and GlcN. The higher growth rate in a medium containing SSH compared to GlcN might be explained by the presence of GlcNAc in the SSH, which leads to the formation of GlcNAc-6-P - an activator for *nagB* (Peri et al., 1990; Alvarez-Añorve et al., 2005).

The substrate-biomass specific yield for all substrates were presented in Table 2. It seems that GlcNAc supports fast growth of strain N5.3 but its low Y_{XS} suggested a higher percentage of the carbon flux was driven to secondary metabolites. On the contrary, the strain grew slower on GlcN but provided higher cell dry weight at the end of the exponential phase, that led to a higher Y_{XS} . The shrimp shell hydrolysate is certainly the most balanced substrate as it provided high μ_{max} as well as Y_{XS} of the tested strains.

Table 2. Growth parameter of *Vibrio natriegens* strains N5.3 on Glc, GlcN, GlcNAc, and SSH at bioreactor scale

Substrate	Y_{XS} (g _{CDW} /g _{Substrate})	μ_{max} (h ⁻¹)	Max CDW* (g.L ⁻¹)
Glc	0.586 ± 0.067	0.792 ± 0.046	9.60 ± 0.23
GlcN	0.669 ± 0.027	0.614 ± 0.035	8.44 ± 0.27
GlcNAc	0.580 ± 0.042	0.881 ± 0.043	7.86 ± 0.12
SSH	0.733 ± 0.035	0.825 ± 0.010	10.56 ± 0.73

Note: *: Value measured at the end of exponential phase.

CONCLUSION

In this work, *V. natriegens* 10.3 and N5.3 strains isolated from Vietnamese seawater and reference strain DSM759 were compared regarding their growth in a medium containing Glc, GlcN, and GlcNAc with 30 g/L NaCl. Consequently, strain N5.3 showed the highest potential to be used as the host for future biotechnology, particularly for the bioconversion of chitin derivatives into molecules of interest.

The growth pattern of strain N5.3 in both flask and bioreactor scales revealed that GlcN

supported high substrate-specific biomass yields but significantly low growth rates while the opposing trend was observed for GlcNAc. This preliminary result suggested that the metabolism of GlcN and GlcNAc in *V. natriegens* may be similar to *E. coli*.

The shrimp shell hydrolysate was proved to be the potential substrate for the cultivation of *V. natriegens* species. The growth rate of strain N5.3 on SSH was comparable to GlcNAc or Glc, and the substrate-specific biomass yield was the highest among all tested substrates.

Acknowledgements: The authors would like to thank the Ministry of Science and Technology for providing funds for project NDT/DE/21/08 to pursue this research. The authors are grateful to Dr. Dam Thuy Hang (School of Chemistry and Life Science, HUST) for the English language review.

REFERENCES

- Alvarez-Añorve L. I., Calcagno M. L., Plumbridge J., 2005. Why does *Escherichia coli* grow more slowly on glucosamine than on N-acetylglucosamine? Effects of enzyme levels and allosteric activation of GlcN6P deaminase (NagB) on growth rates. *Journal of Bacteriology*, 187(9): 2974–2982. <https://doi.org/10.1128/jb.187.9.2974-2982.2005>
- Arnold N. D., Brück W. M., Garbe D., Brück T. B., 2020. Enzymatic modification of native chitin and conversion to specialty chemical products. *Marine Drugs*, 18(2): 93. <https://doi.org/10.3390/md18020093>
- Eagon R. G., 1962. *Pseudomonas natriegens*, a marine bacterium with a generation time of less than 10 minutes. *Journal of Bacteriology*, 83(4): 736–737. <https://doi.org/10.1128/jb.83.4.736-737.1962>
- Eagon R. G., Wang C. H., 1962. Dissimilation of glucose and gluconic acid by *Pseudomonas natriegens*. *Journal of Bacteriology*, 83(4): 879–886. <https://doi.org/10.1128/jb.83.4.879-886.1962>
- Einbu A., Grasdalen H., Vårum K. M., 2007. Kinetics of hydrolysis of chitin/chitosan oligomers in concentrated hydrochloric acid. *Carbohydrate Research*, 342(8): 1055–1062. <https://doi.org/10.1016/j.carres.2007.02.022>
- Ellis G. A., Tschirhart T., Spangler J., 2019. Exploiting the feedstock flexibility of the emergent synthetic biology chassis *Vibrio natriegens* for engineered natural product production. *Marine Drugs*, 17(12). <https://doi.org/10.3390/md17120679>
- Erian A. M., Freitag P., Gibisch M., Pflügl S., 2020. High rate 2,3-butanediol production with *Vibrio natriegens*. *Bioresource Technology Reports*, 10: 100408. <https://doi.org/10.1016/j.biteb.2020.100408>
- Hoffart E., Grenz S., Lange J., Nitschel R., Müller F., Schwentner A., Feith A., Lenfers-Lücker M., Takors R., Blombach B., 2017. High substrate uptake rates empower *Vibrio natriegens* as production host for industrial biotechnology. *Applied and Environmental Microbiology*, 83(22). <https://doi.org/10.1128/aem.01614-17>
- Hou F., Ma X., Fan L., Wang D., Ding T., Ye X., Liu D., 2020. Enhancement of chitin suspension hydrolysis by a combination of ultrasound and chitinase. *Carbohydrate Polymers*, 231: 115669. <https://doi.org/10.1016/j.carbpol.2019.115669>
- Kostag M., El Seoud O. A., 2021. Sustainable biomaterials based on cellulose, chitin and chitosan composites - A review. *Carbohydrate Polymer Technologies and Applications*, 2: 100079. <https://doi.org/10.1016/j.carpta.2021.100079>
- Lee H. H., Ostrov N., Wong B. G., Gold M. A., Khalil A. S., Church G. M., 2019. Functional genomics of the rapidly replicating bacterium *Vibrio natriegens* by CRISPRi. *Nature Microbiology*, 4(7): 1105–1113. <https://doi.org/10.1038/s41564-019-0423-8>
- Li S., You X., Rani A., Özcan E., Sela D. A., 2023. *Bifidobacterium infantis* utilizes N-acetylglucosamine-containing human milk oligosaccharides as a nitrogen source. *Gut Microbes*, 15(2): 2244721. <https://doi.org/10.1080/19490976.2023.2244721>
- Long C. P., Gonzalez J. E., Cipolla R. M., Antoniewicz M. R., 2017. Metabolism of the fast-growing bacterium *Vibrio natriegens* elucidated by ¹³C metabolic flux analysis. *Metabolic Engineering*, 44: 191–197. <https://doi.org/10.1016/j.ymben.2017.10.008>
- Miller G. L., 1959. Use of Dinitrosalicylic Acid Reagent for Determination of

- Reducing Sugar. *Analytical Chemistry*, 31(3): 426–428. <https://doi.org/10.1021/ac60147a030>.
- Payne W. J., 1958. Studies on bacterial utilization of uronic acids. III. Induction of oxidative enzymes in a marine isolate. *Journal of Bacteriology*, 76(3): 301–307. <https://doi.org/10.1128/jb.76.3.301-307.1958>
- Peng Y., Han X., Xu P., Tao F., 2020. Next-generation microbial workhorses: Comparative genomic analysis of fast-growing *Vibrio* strains reveals their biotechnological potential. *Biotechnol J*, 15(5): e1900499. <https://doi.org/10.1002/biot.201900499>
- Peri K. G., Goldie H., Waygood E. B., 1990. Cloning and characterization of the N-acetylglucosamine operon of *Escherichia coli*. *Biochem Cell Biol*, 68(1): 123–137. <https://doi.org/10.1139/o90-017>
- Pfeifer E., Michniewski S., Gätgens C., Münch E., Müller F., Polen T., Millard A., Blombach B., Frunzke J., 2019. Generation of a prophage-free variant of the fast-growing bacterium *Vibrio natriegens*. *Applied and Environmental Microbiology*, 85(17): e00853–00819. <https://doi.org/10.1128/AEM.00853-19>
- Schwarz S., Gerlach D., Fan R., Czermak P., 2022. GbpA as a secretion and affinity purification tag for an antimicrobial peptide produced in *Vibrio natriegens*. *Electronic Journal of Biotechnology*, 56: 75–83. <https://doi.org/10.1016/j.ejbt.2022.01.003>
- Stella R. G., Baumann P., Lorke S., Münstermann F., Wirtz A., Wiechert J., Marienhagen J., Frunzke J., 2021. Biosensor-based isolation of amino acid-producing *Vibrio natriegens* strains. *Metabolic Engineering Communications*, 13: e00187. <https://doi.org/10.1016/j.mec.2021.e00187>
- Thiele I., Gutschmann B., Aulich L., Girard M., Neubauer P., Riedel S. L., 2021. High-cell-density fed-batch cultivations of *Vibrio natriegens*. *Biotechnology Letters*, 43(9): 1723–1733. <https://doi.org/10.1007/s10529-021-03147-5>
- Thoma F., Blombach B., 2021. Metabolic engineering of *Vibrio natriegens*. *Essays Biochem*, 65(2): 381–392. <https://doi.org/10.1042/ebc20200135>
- Thoma F., Schulze C., Gutierrez-Coto C., Hädrich M., Huber J., Gunkel C., Thoma R., Blombach B., 2022. Metabolic engineering of *Vibrio natriegens* for anaerobic succinate production. *Microb Biotechnol*, 15(6): 1671–1684. <https://doi.org/10.1111/1751-7915.13983>
- Wiegand D. J., Lee H. H., Ostrov N., Church G. M., 2018. Establishing a cell-free *Vibrio natriegens* expression system. *ACS Synthetic Biology*, 7(10): 2475–2479. <https://doi.org/10.1021/acssynbio.8b00222>
- Zhang Y., Li Z., Liu Y., Cen X., Liu D., Chen Z., 2021. Systems metabolic engineering of *Vibrio natriegens* for the production of 1,3-propanediol. *Metabolic Engineering*, 65: 52–65. <https://doi.org/10.1016/j.ymben.2021.03.008>