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Hydrolysis of Chitin with Acid and Noble Metal Catalyst

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Abstract: Chitin, abundant in shrimp and crab shells, can be hydrolyzed to produce glucosamine, a compound widely used in medicine, agriculture, and material science. Efficient production of chitin monomers and their derivatives on a large scale can enhance the utilization of marine resources and reduce environmental pressure. However, current hydrolysis methods face technological and cost-related challenges, limiting them to experimental or small-scale production. This study explores the hydrolysis of chitin using acids, salts, and activated-carbon-supported transition metals, focusing on optimizing reaction parameters for improved monomer yield. Among six acids tested, hydrochloric acid yielded the highest amount of N-acetyl-D-glucosamine (NAG), while sulfuric acid was most effective for glucosamine (GlcN) production. Palladium-carbon was the most efficient metal catalyst for NAG yield, while nickel powder catalyzed the highest GlcN production. It was observed that increasing chitin quantity, while keeping phosphoric acid constant, led to a decrease in NAG and GlcN yields. Temperature variations within the range of 100-120 ℃ showed minimal impact on the hydrolysis results. Additionally, among three salt catalysts, ammonium formate and ammonium acetate were most effective for NAG and GlcN yields, respectively. These methods offer potential for preparing chitin monomers or their derivatives while reducing energy consumption and simplifying hydrolysis conditions.

Keywords: Valorization of Biomass; Hydrolysis of Chitin; Catalytic Conversion; N-acetyl-D-glucosamine; Glucosamine

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1 Introduction

Marine biomass, including seaweed, microalgae and marine organisms, is rich in resources and has broad prospects for development and utilization [1]. High-value utilization of marine biomass can reduce dependence on traditional energy resources [2], help reduce environmental pollution and ecological damage [3], and meet the requirements of sustainable development [4, 5]. The use of marine biomass to produce biofuels [6–11], bioplastics [12], and other products can not only reduce energy costs [13, 14], but also develop new economic growth points and promote sustainable economic development [15–17]. High-value utilization needs to involve research and development in many fields, such as biotechnology, materials science, environmental science, etc., to promote technological progress and innovation [18–20].

Marine resources are abundant [21]. Chitin is an important component of marine biomass, mainly found in the shells of crustaceans (e.g. shrimp, crab, lobster) [22]. High-value utilization of chitin has important application prospects and significance, specifically in the field of bi-

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omedicine [23], agriculture [24], food industry [25], environmental protection [26], material science [27] and so on. The multifunctionality and wide application prospects of chitin make it an important target for high value utilization of marine biomass [28]. Through technological innovation and industrialization development, the utilization of chitin will bring significant social and economic benefits to the fields of medicine, agriculture, food, environmental protection and materials [29, 30].

The monomer NAG of chitin, which is widely found in shrimp and crab shells and arthropods [31], has a broad application prospect in chemical industry [32], medicine [18], agriculture [33], daily necessities, food [34] and environmental protection [26]. So far, we are still committed to discovering more efficient catalytic systems and materials to increase the selectivity of hydrolysis and more efficiently carry out the hydrolysis of chitin into monomers [35–37], to promote the high-value utilization of marine chitin.

In this paper, an attempt has been made to study the effect of reaction parameters such as type of catalyst, substrate concentration, and temperature on the yield of NAG and GlcN from the hydrolysis reaction of chitin for acid hydrolysis.

2 Experimental Section

2.1 Experimental Reagents

The names, chemical formulas, specifications, and manufacturers of the reagents used during the experiment are listed in Table 1.

Table 1 Experimental reagents

materials	**formula**	**purity**	**company**
chitin	$(C_8H_{13}O_5N)_n$	A.R.	Sigma Biotechnology Ltd.
N-acetyl-D-glucosamine	$C_8H_{15}NO_6$	A.R.	Shanghai Aladdin Biochemicals Co. Ltd
Phosphoric acid	H_3PO_4	A.R.	Tianjin Damao Chemical Reagent Factory
Activated carbon	C	A.R.	Shanghai macklin Biochemical Technology Co., Ltd.
Pt/C			Shanghai macklin Biochemi-cal Tech-nology Co., Ltd.
Pd/C			Shanghai macklin Biochemical Technology Co., Ltd.
Ni			Shanghai macklin Biochemical Technology Co., Ltd.
Sulfuric acid	H_2SO_4	A.R.	Guangzhou Chemical Reagent Factory
Ammonium acetate	$C_2H_7NO_2$	A.R.	Guangzhou Chemical Reagent Factory
Formic acid	CH_2O_2	A.R.	SAS Chemical Technology (Shanghai) Ltd.
Acetic acid	$C_2H_4O_2$	A.R.	Guangzhou Chemical Reagent Factory
Citric acid	$C_6H_8O_7$	A.R.	Shanghai Aladdin Biochemicals Co. Ltd
Hydrochloric acid	HCl	A.R.	Guangzhou Chemical Reagent Factory
Ammonium formate	CH_5NO_2	A.R.	Shanghai Boer Chemical Reagent Co. Ltd.
Dipotassium chloride	KCl	A.R.	Shanghai Boer Chemical Reagent Co. Ltd.

2.2 Hydrolysis Procedure

The expected amount of water and acid were added to a 35 mL glassware; the chitin was aged in the glassware for 24 h, so that the chitin was dissolved, and then the glassware was put into an oil bath and the rotational speed of the magnetic stirrer was adjusted to 500 r/min for the reaction for 24 h. The structure of chitin and acid-dissolved chitin were characterized by infrared spectroscopy, and the structural effects of the chitin type and the acid on the chitin were determined, and then the hydrolysis solution (containing dissolved chitin oligomers) was obtained using different reaction conditions, and the products were detected by liquid chromatography (HPLC) and data processing. Then, different reaction conditions were used to obtain the hydrolysate (containing dissolved chitin oligomers), and the products were detected by liquid chromatography (HPLC) and data processing, and the contents of chitin monomers NAG and GlcN were determined more accurately.

In order to further investigate the effect of reaction parameters on the yield, the time, temperature, substrate concentration, salt concentration and other parameters of the hydrolysis of chitin reaction were optimized, and the catalyst was unified with ammonium formate, the mass of chitin was aged according to the multiplicity treatment (1-fold, 2-fold, 4-fold, 6-fold, 8-fold, 10-fold) for 24 h, and then the reaction was carried out in a constant-temperature oil bath with the rotational speed of the magnetic stirrer adjusted to 500 r/min for 24 h. The reaction was carried out according to the above method, and the reaction was carried out according to the above method. The reaction was carried out for 24 h. The structural characterization analysis was completed and the yield was determined according to the qualitative and quantitative methods mentioned above, and the change rule of the yield was observed.

2.3 HPLC Analysis

Liquid chromatography conditions: Agilent 1260 chromatography system was used. The Agilent 1260 HPLC mainly includes a quaternary pump, an autosampler, a column temperature chamber, a VWD, a RID, and a chemical workstation, with a detection wavelength of 254 nanometers; a column temperature of 40 degrees Celsius; a flow rate of 0.6 milliliters per minute; a mobile phase: solvent 100% H_2O; and an injection volume of 5 microliters. When using the Agilent 1260 HPLC, replace the mobile phase with a clean one and clean the filter in time to improve the operational performance of the pump.

Sample characterization and quantification: 1 ml of the experimentally obtained hydrolysate was aspirated with a 1 ml syringe and filtered through a 22 μm nylon microporous membrane to ensure that the sample was free of solid particulate matter before placing it in a vial ready for injection. The product was characterized by comparing the peak times of NAG and GlcN standards, and quantified by the standard curve prepared. The oligomeric package peaks appeared in the spectra due to the fact that complex mixtures of depolymerization products with different numbers of repeating units are generated during the hydrolysis of chitin to monomeric NAG.

The working curves between mass concentration and peak area of the standards were established as shown in Figures 3 and 4. From the peak times of the standards (3.5-4.5 min for GlcN and 5.1-5.85 min for NAG), the peak positions of the products were compared in the result plots from the RID detector and the VWD detector in order to confirm the corresponding output fractions, and the peak areas of the products were obtained by the manual integration operation, and then according to the peak area-mass concentration regression of The mass concentrations of the components GlcN (Figure 1) and NAG (Figure 2) for this experiment were calculated from the regression equations Y=1322.3570x and Y=990.5859x, which in turn gave the yields of these two products.

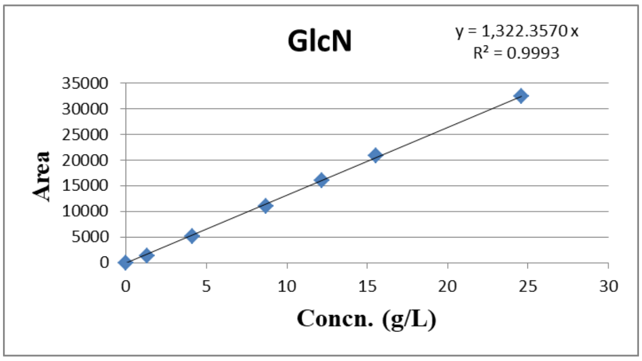

Figure 1 Standard curve of GlcN

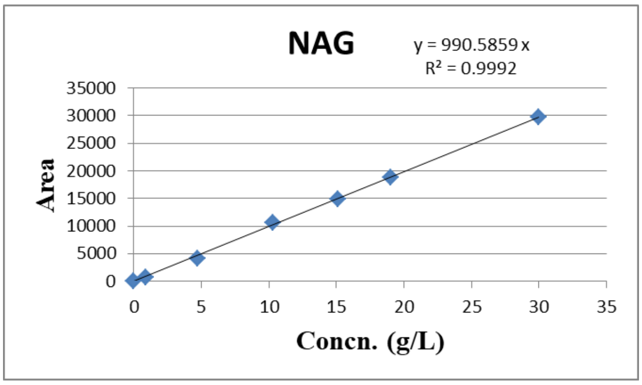

Figure 2 Standard curve of NAG

3 Results and Discussion

3.1 Metal Catalyst

Generally, the catalytic reaction will take place at the atoms located on the surface, so the carrier metal catalyst with good dispersion of the active metal on the carrier surface will have better activity, selectivity and stability, and it will have a stronger level of resistance to carbon buildup and resistance to metal sintering. The high dispersion of the metal also enables the efficient utilization of the metal, reducing the amount of metal used and the expense of the catalyst in production. The multiphase metal catalysts used in this paper are palladium carbon catalysts, platinum carbon catalysts, and nickel powder, in which Pt and Pd loaded on the active carbon are highly dispersed with a large active surface and combined with the different properties of the two materials, they have a strong resistance to acids and alkalis and high porosity.

As shown in Figure 3, observing the effect of metal-catalyzed reaction activity, through the analysis of the hydrolysis of chitin phosphate to produce GlcN at a specific temperature, it can be concluded that: three metal catalysts (platinum carbon, palladium carbon and nickel powder), nitrogen-doped activated carbon and activated carbon catalysts compared to the catalysts are able to increase the yield, and the catalytic hydrolysis of the catalysts, the effect of the catalysts are in the following order: nickel powder>nitrogen-doped activated carbon>platinum carbon catalyst > palladium carbon catalyst > activated carbon; in comparing the yield of NAG, the catalytic effect of metal catalysts was ranked as palladium carbon catalyst > nickel powder > activated carbon > platinum carbon catalyst and nitrogen-doped activated carbon.

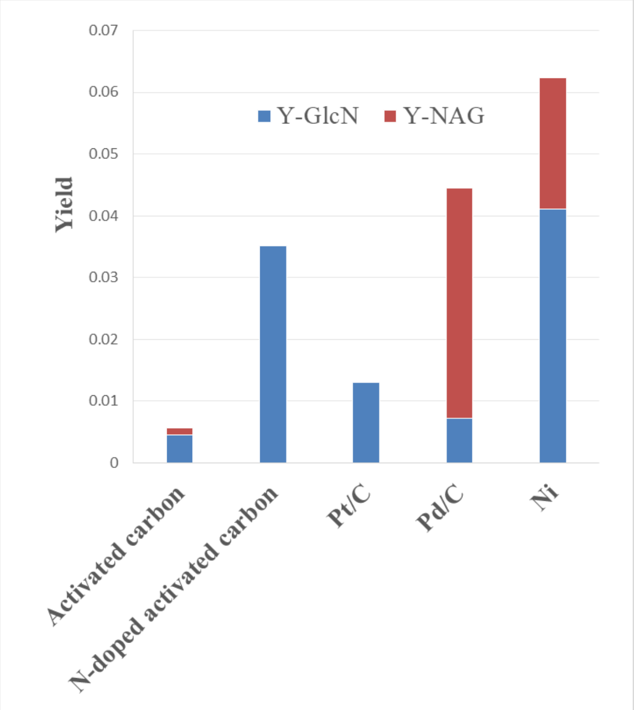

Figure 3 Comparison of catalysts after phosphoric acid aging

3.2 Acid Catalytic Method

The acid hydrolysis of chitin to monomers by chemical methods requires high temperatures (higher than 120 degrees Celsius), in the present work an attempt was made to hydrolyze chitin at a low temperature of 80 degrees Celsius with various organic and inorganic acids. The effect of different acids on the yield of the hydrolysis reaction was observed as shown in Figure 4. From the yield of monomer GlcN, the catalytic effect was ranked as, sulfuric acid > formic acid > hydrochloric acid > phosphoric acid > citric acid; while the effect of selective hydrolysis of chitin to monomer NAG was ranked as hydrochloric acid > acetic acid > formic acid > phosphoric acid > citric acid and sulfuric acid, as shown in Figure 4.

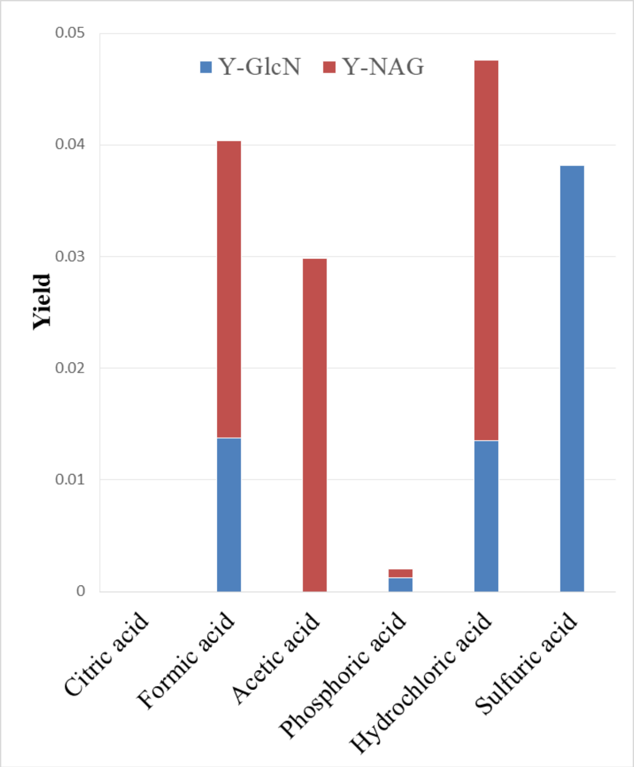

Figure 4 Effect of different acid hydrolysis on yields of GlcN and NAG at low temperature

3.3 Salt-catalyzed Method

In recent years, much attention has been paid to the research and development of adding metal ions as co-catalysts in acid hydrolysis. With relevant research, scientists have observed that in addition to C, H, O, there are also small amounts of inorganic salt elements, such as potassium, sodium, calcium, iron, copper and magnesium, in the biomass feedstock. These elements, although in very small amounts, show important catalytic roles in the chemical conversion of biomass, thus receiving more and more attention. In the experimental hydrolysis of chitin to monomeric NAG with potassium chloride catalyzed by phosphoric acid, the acid-induced K^+ cations and Cl^- anions strongly interact with the chitin chain by coordination with O atoms on C=O, NH_2 or by formation of hydrogen bonds with hydroxyl groups, respectively, which leads to the selective generation of NAG by the further rupture of glycosidic bonds. In the present experiments, NAG was selectively generated by further breaking of the glycosidic bonds with the hydrolysis of chitin with phosphoric acid only, it was verified that the use of the metal salt KCl catalyst did act positively on the conversion process. In addition, in combination with the fact that all ammonium salt catalysts react with cellulose at elevated temperatures to generate organic amine salts for catalytic purposes, the use of ammonium salts as catalysts catalyzed the hydrolysis of chitin to monomer reaction. The catalytic effect of the three salt catalysts used in the experiment, in terms of the yield of monomer GlcN, was ranked as, ammonium acetate > potassium chloride > ammonium formate; while the effect on the selective hydrolysis of chitin to monomer NAG was ranked as ammonium formate > potassium chloride > ammonium acetate, as shown in Figure 5.

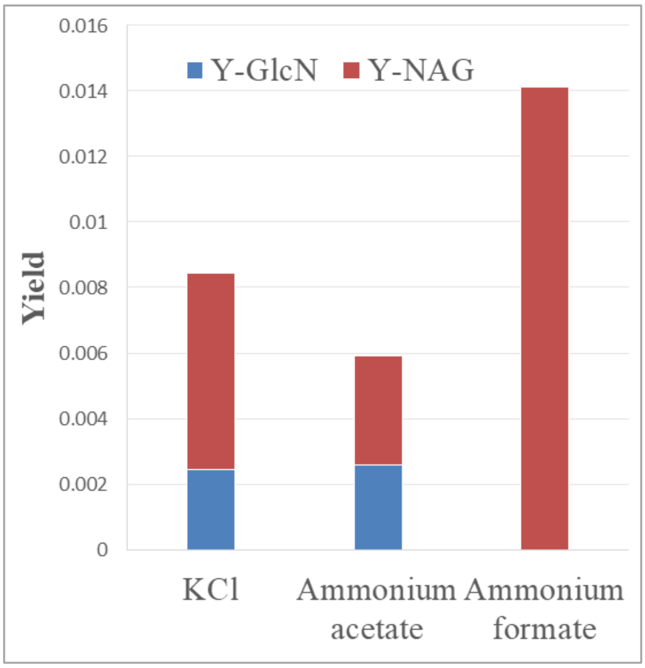

Figure 5 Effect of different salts on yields of GlcN and NAG

Based on the ammonium formate-catalyzed hydrolysis of chitin by phosphoric acid, the hydrolysis experiments were carried out by reducing the mass of the reactant chitin by multiplicative treatments (1x, 2x, 4x, 6x, 8x, and 10x), and the mass of water and phosphoric acid of each multiplicative-treated experiment was reduced to one-fifth of the original mass and the hydrolysis experiments were carried out, and the changes in the yields of both GlcN and NAG could be observed, as shown in Figure 6. Because the mass of phosphoric acid was controlled to remain constant, the ability of phosphoric acid to age chitin remained constant, and as the mass of chitin increased, so the yields of the products GlcN and NAG both became smaller and smaller.

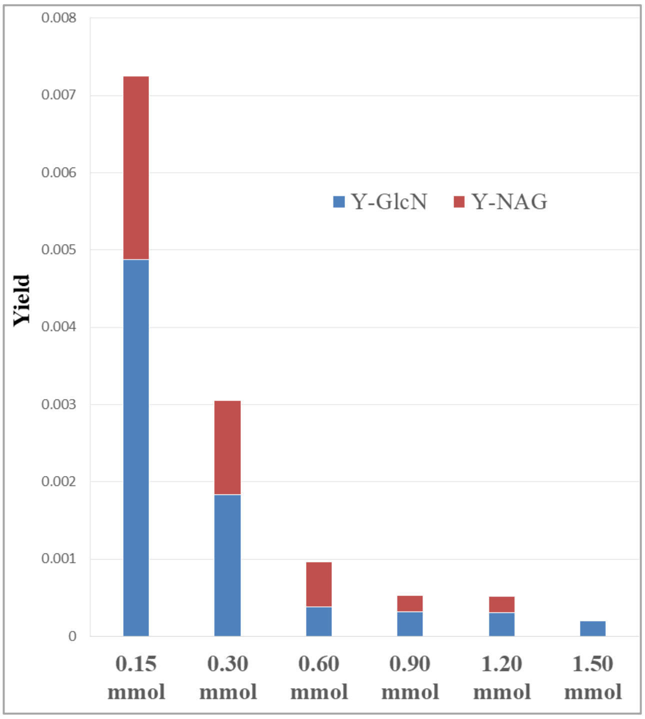

Figure 6 Effect of different substrate concentrations on yields of GlcN and NAG

On the basis of ammonium formate-catalyzed hydrolysis of chitin by phosphoric acid, the effect of temperature change on the hydrolysis reaction of chitin was investigated, as shown in Figure 7. It was found that the experimental results did not change significantly after the temperature was operated in the interval of 100-120 ℃ for warming.

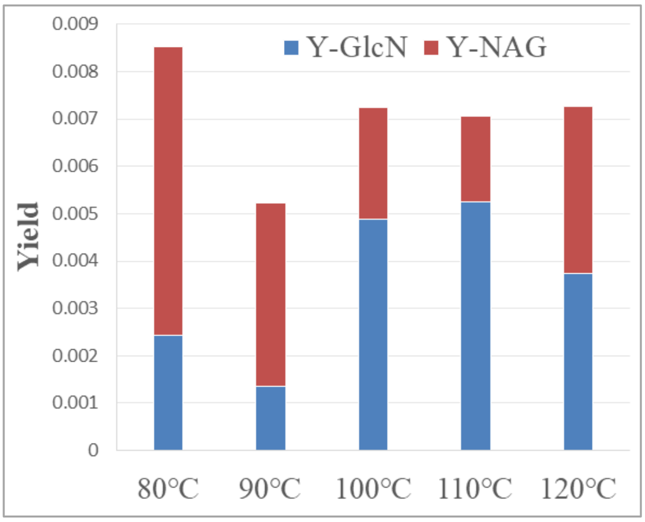

Figure 7 Effect of temperature on yields of GlcN and NAG

4 Conclusion

(1) To compare the order of the effect of different metal catalysts on the hydrolysis reaction, the yield of NAG was highest with palladium-carbon catalysts, followed by nickel powder and platinum-carbon catalysts, while the yield of GlcN was highest with nickel powder, followed by platinum-carbon catalysts and palladium-carbon catalysts.

(2) Comparison of the order of effect of different acid treatments on the hydrolysis reaction. Hydrochloric acid treatment had the greatest effect on the yield of NAG, followed by acetic acid, formic acid, phosphoric acid, citric acid and sulfuric acid; while sulfuric acid had the greatest effect on the yield of GlcN, followed by formic acid, hydrochloric acid, phosphoric acid and citric acid.

(3) Compare the order of effect of different substrate mass concentrations on the hydrolysis reaction. At a constant mass of phosphoric acid, the yields of both NAG and GlcN become smaller and smaller as the mass of chitin increases.

(4) A comparison of the effects of different temperature conditions on the yield of the hydrolysis reaction revealed that variations in experimental results were minimal, and the selectivity of the hydrolysis reaction was not significantly impacted when the temperature was increased within the range of 100-120 ℃.

(5) Comparison of the effect of using salt as catalyst on the hydrolysis reaction. For the three salt catalysts used in the experiment, the yield of NAG was highest under ammonium formate catalyst, followed by potassium chloride and ammonium acetate; while the yield of GlcN was highest under ammonium acetate catalyst, followed by potassium chloride and ammonium formate.

(6) Future catalyst designs should focus on improving the catalyst's efficiency, selectivity, reusability, and ability to operate under mild reaction conditions. Currently, fewer chemicals have been derived from chitin biomass compared to those produced from cellulose. Therefore, it is necessary to develop additional reaction pathways for chitin biomass. The applications of the chemicals identified so far are limited and mostly remain at the laboratory research stage. Considerable effort is required to explore the potential uses of these chemicals in advancing shell biorefinery.

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