NOVOZYMES' CELLULOSIC ETHANOL ENZYME KIT

– Enzymes for the hydrolysis of lignocellulosic materials

novozymes®
Bioenergy

ENZYMES FOR THE HYDROLYSIS OF LIGNOCELLULOSIC MATERIALS

You have received the Novozymes Cellulosic Ethanol Enzyme Kit. This document contains useful information designed to help you understand more about cellulosic ethanol production and how our innovative enzymes can optimize your plant processes and enable commercialization.
Following this brief overview of our work with cellulosic ethanol, you will find basic application information regarding the use of the enzymes contained in this sample kit. More specific details about the characteristics, activity, dosage, etc. of each enzyme are included in the Appendices beginning on page 6.

Overview

Novozymes leads the way in cellulosic ethanol through broad partnerships that employ many feedstocks and processes. We provide the industry's best enzyme solutions and process optimizations to enable commercialization with partners and in our own labs.

We take a holistic approach in developing next-generation bioinnovations to meet your company's ever-changing needs. Along with our unprecedented R&D efforts, we also work closely with our partners to bring different competencies together in an effort to fully understand the critical relationship between process mechanics and enzymes. Together we can create solutions that realize the promise of renewable energy.

Cellulosic ethanol can be a major source of sustainable energy. Many feedstocks – including corn cobs, wheat straw, woody biomass, and municipal solid waste – are readily available, and it is estimated that cellulosic ethanol will reduce CO_2 emissions by 90% compared to petroleum-based fuels.

The complex structure of biomass make it more difficult to convert into ethanol than traditional starch substrates. This has presented unique technical and economic challenges in bringing cellulosic ethanol to market. Enzymes are vital in the conversion of biomass to ethanol, and the state-of-the-art Novozymes Cellic® solutions make the technology available at a commercially viable cost. Our groundbreaking innovation is a result of our commitment to creating sustainable solutions that improve the environment and enhance your business.

We are working every day to develop technologies that allow more types of biomass to be turned into commercially viable biofuel.

Our biomass test kit provides you with our leading enzymes for enabling the conversion of a variety of cellulosic feedstocks.

Application

In order to maximize the yield from enzyme hydrolysis, a combination of enzyme activities must be used. The optimal enzyme blend greatly depends on the composition of the various fractions (cellulose, hemicellulose, and lignin) in the biomass substrate. Experiments should be conducted to determine which enzymes and pretreatment method will work best on a specific feedstock.

The following tables contain information on the enzymes included in the test kit. The suggested enzyme dosage is based on weight percentage relative to the amount of biomass (total solids) on a dry basis. The required enzyme dosage may vary significantly based on the specific composition of the biomass feedstock and the particular physical and/or chemical pretreatment method used. Even though some of the enzymes have high temperature ranges, they will still work at lower temperatures, albeit at reduced activity. Operating at temperatures above the upper limit can result in irreversible enzyme denaturation. The general impact of temperature on enzyme activity and stability is shown in Appendix B.

Find out more at
www.bioenergy.novozymes.com

NS number	Enzyme type	Description
NS22086	Cellulase complex	- Primary enzyme for use in the hydrolysis of lignocellulosic material - Catalyzes the breakdown of cellulosic material into glucose, cellobiose, and higher glucose polymers - Can be used to reduce the viscosity or increase the extraction yield of various products of plant origin - The main reaction products of cellulose hydrolysis using NS22086 are cellobiose and glucose - Testing for synergy with NS22118 and NS22083 is recommended to maximize performance
NS22083	Xylanase	- Purified endoxylanase with a high specificity toward soluble pentosans - Able to liberate pentose sugars from biomass hemicellulose fractions - Can be used to supplement NS22086 for pretreatment that leaves a significant portion of the hemicellulose intact (i.e., neutral-pH or alkaline pretreatment methods)
NS22118	β-glucosidase	- Also known as cellobiase; hydrolyzes cellobiose to glucose - Can be used to supplement NS22086 in order to increase the yield of fermentable sugars - Addition should be approximately 0–4% (v/v) of the amount of NS22086 for complete hydrolysis of the available cellulose
NS22119	Enzyme complex	- Contains a wide range of carbohydrases, including arabinase, β-glucanase, cellulase, hemicellulase, pectinase, and xylanase - Can break down cell walls for the extraction of useful components from plant tissue - Able to liberate bound materials and degrade a variety of nonstarch polysaccharides - Can be used to supplement NS22086 for substrates containing pectin
NS22002	β-glucanase Xylanase	- Contains a mixture of β-glucanase and xylanase enzyme activities - Possesses additional side activities, including cellulase, hemicellulase, and pentosanase - Can be used to supplement NS22086 for pretreatment that leaves a significant portion of the hemicellulose intact (i.e., neutral-pH or alkaline pretreatment methods)
NS22035	Glucoamylase	- Used on liquefied starch-containing substrates to produce sugars for fermentation - Works in dedicated saccharification stages as well as simultaneous saccharification and fermentation - Glucoamylases hydrolyze both 1,4- and 1,6-alpha linkages to liberate glucose for subsequent fermentation by the yeast

Table 1. Descriptions of enzymes contained in Novozymes' cellulosic ethanol enzyme kit.

Enzyme classification	Activity[1]	Density[2] (g/ml)	pH	Temperature (°C)	Dosage[3] (% w/w (TS))
NS22086 Cellulase complex	1,000 BHU(2)/g	1.15	5.0–5.5	45–50	1–5%
NS22083 Xylanase	2,500 FXU-S/g	1.09	4.5–6.0	35–55	0.05–0.25%
NS22118 β-glucosidase	250 CBU/g	1.2	2.5–6.5	45–70	0.2–0.6%
NS22119 Enzyme complex	100 FBG/g (~ 13,700 PGU/g)	1.19	4.5–6.0	25–55	0.05–0.4%
NS22002 Hemicellulase	45 FBG/g (~ 470 FXU/g)	1.20	5.0–6.5	40–60	0.4–2%
NS22035 Glucoamylase	750 AGU/g	1.15	4.5–5.5	60–70	0.01–0.06%

Table 2. Enzyme activity, density, pH, temperature, and recommended dosage.

1) EGU = Endo-Glucanase Unit, CBU = CelloBiase Unit, FBG = Fungal Beta-Glucanase Unit, PGU = PolyGalacturonase Unit, FXU-S = Fungal Xylanase Unit, and AGU = AmyloGlucosidase Unit. See Appendix A for further information on activity units.

2) Density values are approximate.

3) The required dosage is heavily dependent on feedstock type, pretreatment technology, and processing conditions. Enzyme dosage requirements may therefore vary significantly.

Pretreatment conditions must be optimized to achieve the maximum conversion of polysaccharides to fermentable sugars while minimizing the enzyme requirement.
It is essential to evaluate the pretreated biomass for cellulose digestibility at an appropriate solids concentration.

If the solids concentration in a hydrolysis experiment is too high, nonenzymatic factors can be introduced that will interfere with the interpretation of the results. A range of 2–5% total solids (TS) loading is suggested for determining the efficacy of the pretreatment system. Results can be compared by evaluating the required enzyme dosage per mass of cellulose in the feedstock (cellulose content can be determined chemically) in order to give an indication of the enzymatic digestibility of the pretreated substrate (i.e., the efficiency of the pretreatment technology).

Note: Inhibition of enzymatic hydrolysis

The pretreatment method can create inhibiting products that can reduce the performance of the enzyme, resulting in lower cellulose conversion and/or increased enzyme dosage. Lignin and xylo-oligomer released during the pretreatment process can interfere with enzymatic performance by binding irreversibly to the enzymes or by blocking enzyme access to the substrate. An optimal degree of pretreatment does exist where the carbohydrate enzyme accessibility is maximized while the enzyme (and microorganism) inhibition is minimized.

From a cost perspective, it is normally advantageous to perform hydrolysis at the highest possible total solids in order to achieve maximum sugar concentrations.

However, the higher solids conditions can also mean higher concentrations of soluble compounds in the liquid phase, which may impede enzyme activity. The required enzyme dosage to satisfactorily hydrolyze a substrate is thus a factor of both the accessible cellulose and the relative concentration of inhibitory compounds. It is worthwhile considering how best to simulate your desired process system concentrations relative to the enzyme protein concentration during your evaluation with this biomass kit.

Additional information

We invite you to explore our biomass information site at www.bioenergy.novozymes.com.

Disclaimer

The enzymes provided in this cellulosic ethanol enzyme kit should be used only for the conversion of lignocellulosic materials and should not be used with other materials that are to be used in food.

Risks may be associated with this process, and Novozymes and our affiliated companies and their respective employees, affiliates, and agents will not be liable in respect of any claims that may arise as a result of the materials contained in this cellulosic ethanol enzyme kit.

In addition, NOVOZYMES EXPRESSLY DISCLAIMS ALL WARRANTIES, EXPRESS OR IMPLIED, INCLUDING WITHOUT LIMITATION WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, AND NONINFRINGEMENT OF THIRD-PARTY INTELLECTUAL PROPERTY RIGHTS.

Appendix A: Activity units and measurements

The following table contains information on the various enzyme activities that are contained in Novozymes' cellulosic ethanol enzyme kit. For additional information on enzyme activity analyses, please contact Novozymes using the contact form at www.bioenergy.novozymes.com to place your inquiry.

Abbreviation	Description	Definition
AGU	AmyloGlucosidase Unit	Amyloglucosidase activity in AGU is measured relative to a Novozymes AGU enzyme standard
CBU	CelloBiase Unit	One CBU is the amount of enzyme that releases 2 µmol glucose per minute under standard conditions with cellobiose as substrate
BHU(2)	Biomass Hydrolysis Unit	A Biomass Hydrolysis Unit (BHU(2)) measures the enzyme activity needed to hydrolyze cellulose that is present in a complex biomass substrate under the conditions given in this method. The activity is determined relative to an enzyme standard
FBG	Fungal Beta-Glucanase Unit	One FBG is the amount of enzyme that produces reducing carbohydrate equivalent to 1 µmol of glucose per minute under the conditions given in this method. The activity is determined relative to an enzyme standard
FPU	Filter Paper Unit	0.185 FPU is the quantity of enzyme activity that, when assayed according to the standard FPU method, produces reducing sugar equivalent to 2.0 mg of glucose Reference: http://www.nrel.gov/biomass/pdfs/4689.pdf
FXU	Fungal Xylanase Unit	Endoxylanase activity in FXU-S is measured relative to a Novozymes FXUS enzyme standard
PGU	Polygalacturonase Unit	PolyGalacturonase activity in PGU is measured relative to a Novozymes enzyme standard

Table 3. Description of activity units.

Appendix B: Activity and stability response curves

NS22086

Activity and stability

Figures 1 and 2 illustrate the activity of NS22086 at different temperatures and pH values using washed, dilute acid-pretreated corn stover as substrate. The pH and thermostability of the enzyme in aqueous solutions can be seen from Figures 1 and 2. For practical applications the optimal conditions are about 45–50 °C (113–140 °F) and pH 5.0.

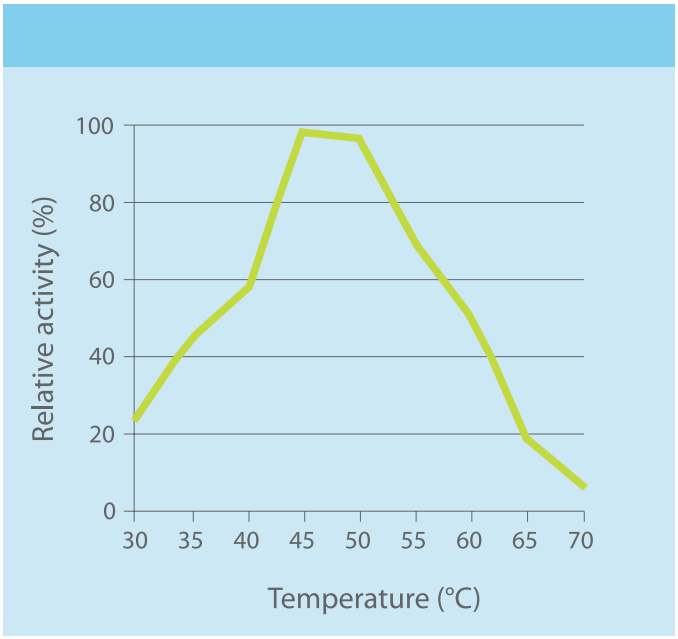

Fig. 1: Relative activity of NS22086 as a function of temperature (°C) at pH 5.0 using washed, dilute acid-pretreated corn stover.

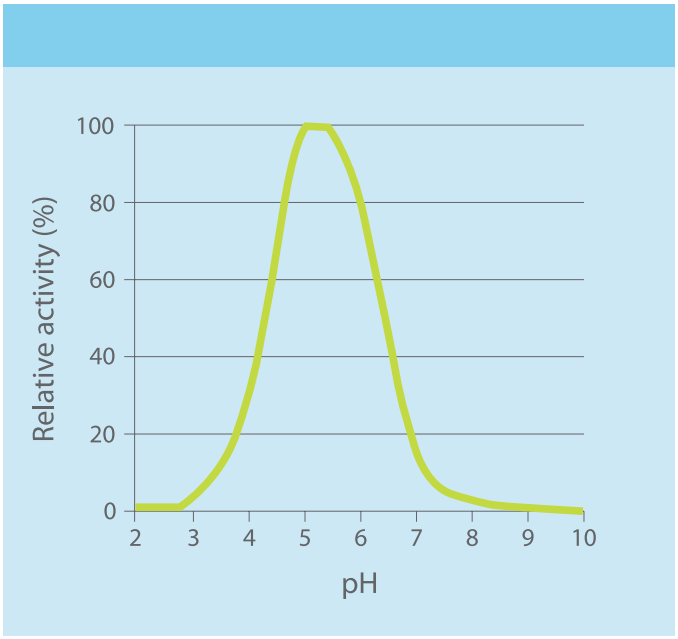

Fig. 2: Relative activity of NS22086 as a function of pH at 50 °C using washed, dilute acid-pretreated corn stover.

NS22083

Activity and stability Figures 3 and 4 illustrate the activity of NS22083 at different temperatures and pH values using azo-wheat arabinoxylan as substrate. The heat and pH stability of the enzyme in aqueous solutions can be seen from Figures 5 and 6. For practical applications the optimal conditions are about 35–55 °C (95–131 °F) and pH 4.5–6.0.

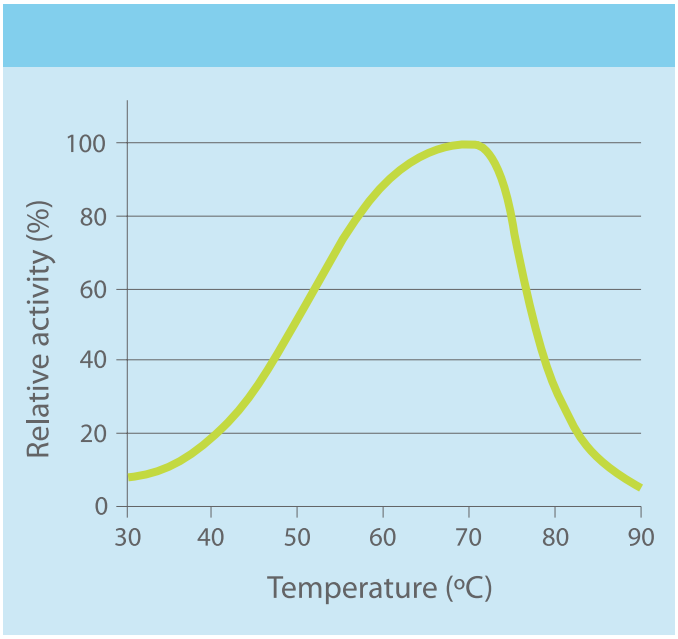

Fig. 3: Effect of temperature on the activity of NS22083.
Substrate: Azo-wheat arabinoxylan
pH: 4.0
Reaction time: 10 min

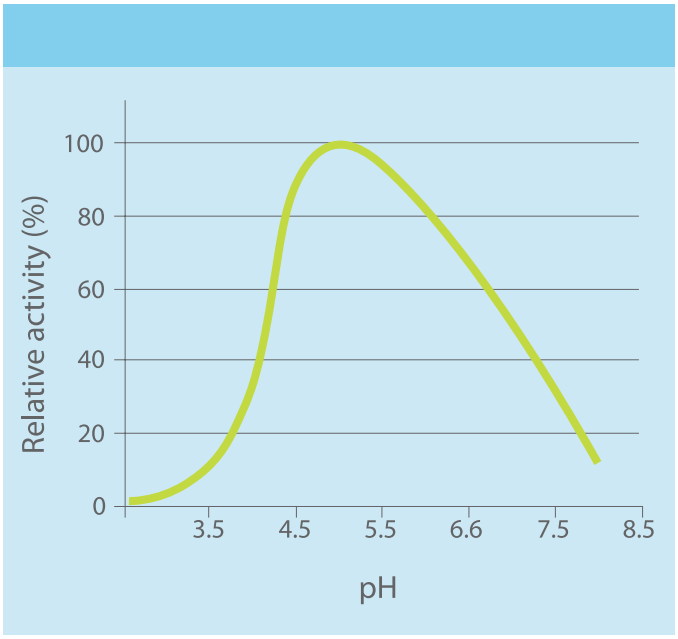

Fig. 4: Effect of pH on the activity of NS22083.
Substrate: Azo-wheat arabinoxylan
Temperature: 70 °C
Reaction time: 10 min

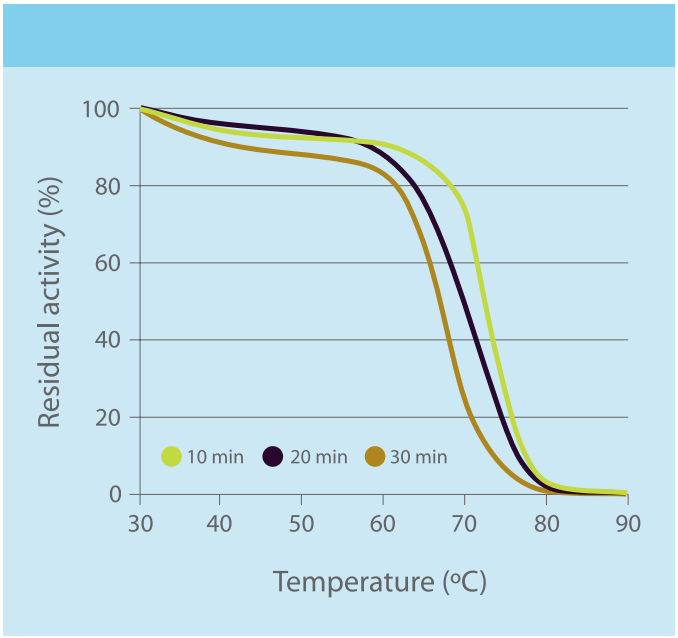

Fig. 5: Effect of temperature on the stability of NS22083.
Substrate: Azo-wheat arabinoxylan
pH: 4.5

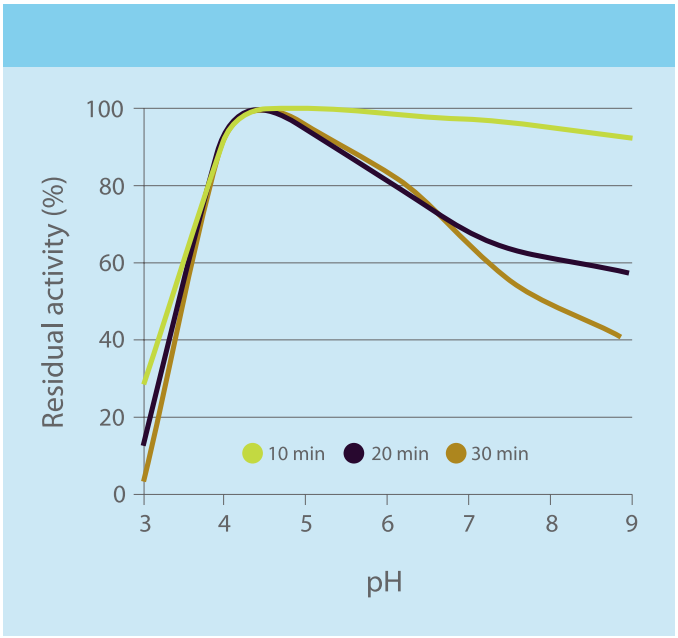

Fig. 6: Effect of pH on the stability of NS22083.
Substrate: Azo-wheat arabinoxylan
Temperature: 50 °C (122 °F)

NS22119

Description

NS22119 is a multienzyme complex containing a wide range of carbohydrases, including arabinase, β-glucanase, cellulase, hemicellulase, pectinase, and xylanase. Its main activities are polygalacturonase, mannanase, and β-glucanase. In addition, it contains a range of plant cell wall-degrading enzymes such as pectin lyase, pectin esterase, and rhamnogalacturonase. It also contains some galactanase and other hemicellulolytic activities. The enzyme preparation is produced from a selected strain of *Aspergillus aculeatus*. Optimal conditions for NS22119 are pH 4.5–6.0 and 25–55 °C.

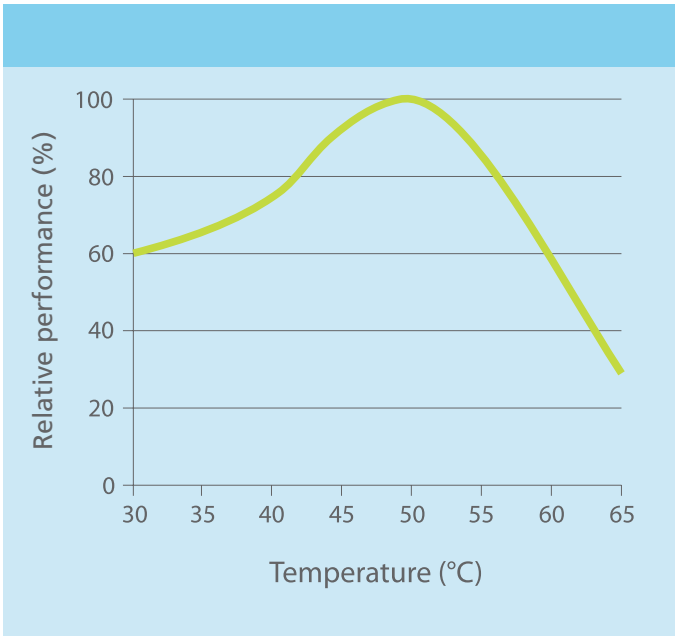

Fig. 7: The effect of temperature on the activity of NS22119.

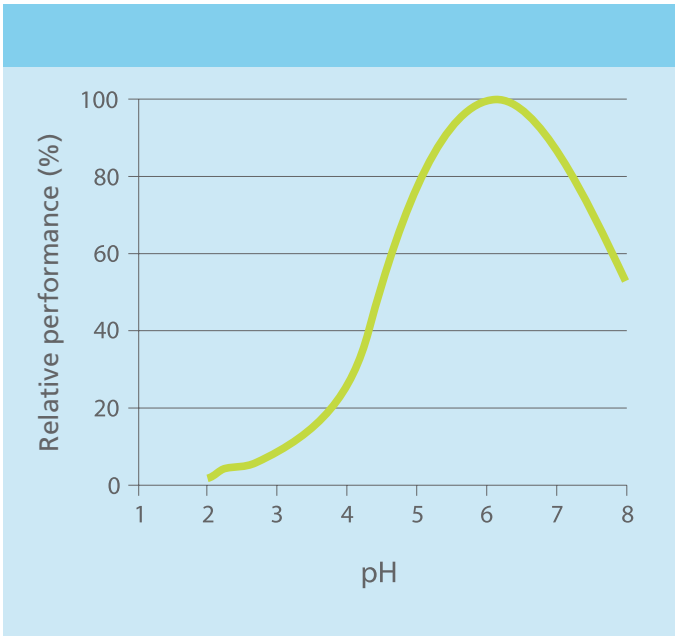

Fig. 8: The effect of pH on the activity of NS22119.

NS22002

Description

NS22002 is a mixture of β-glucanase and xylanase enzymes produced by submerged fermentation of a *Humicola insolens* strain. β-glucanase and xylanase are the two main enzyme activities in the preparation, but the product also contains several other side activities, including cellulase, xylanase, arabinase, and pentosanase. Optimal conditions for NS22002 are pH 5.0–6.5 and 40–60 °C.

Product type

NS22002 is a brown liquid with a density of approximately 1.2 g/ml.

Product activity

NS22002 has a typical standardized activity of 45 FBG/g, and in addition it has approximately 470 FXU/g.

NS 22035

Activity

The relative performance of NS22035 at varying temperature and pH is shown in Figures 9 and 10. The testing was conducted using corn mash at 29% solids at 65 °C for 24 hours. The pH and temperature optima are considered to be 4.5–5.5 and 60–70 °C respectively.

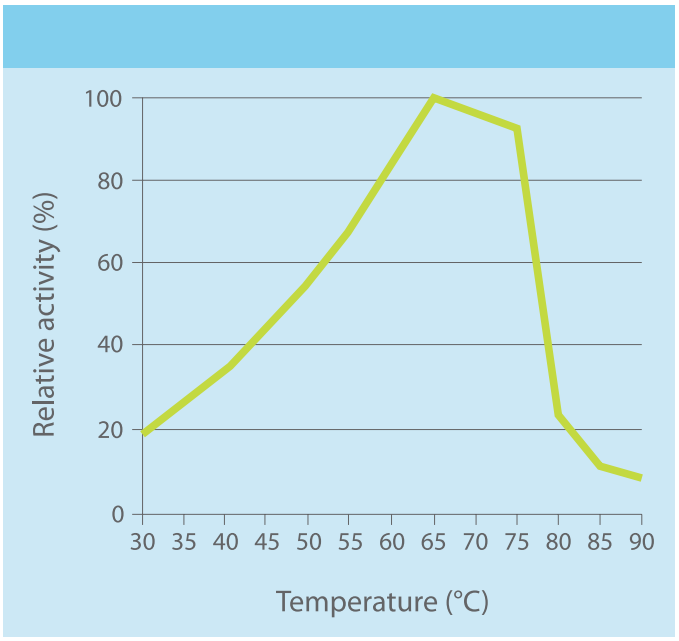

Fig. 9: The effect of temperature on the acticvity of NS 22035.

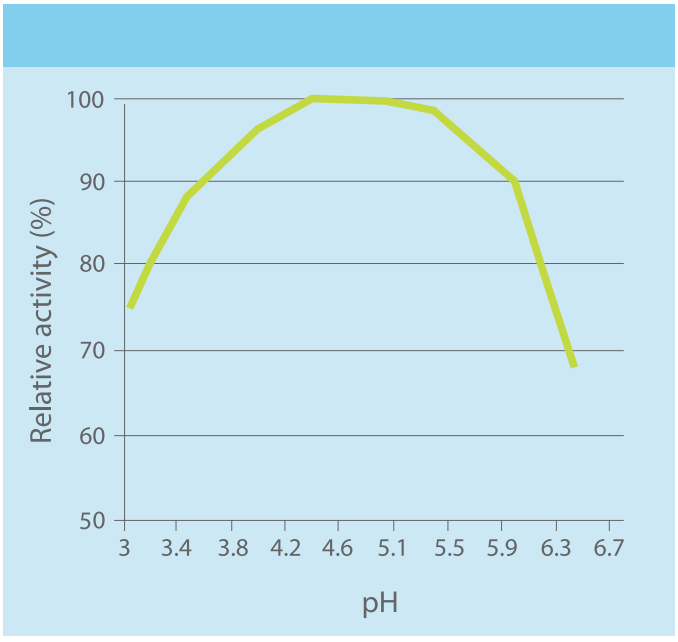

Fig. 10: The effect of pH on the activity of NS 22035.

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