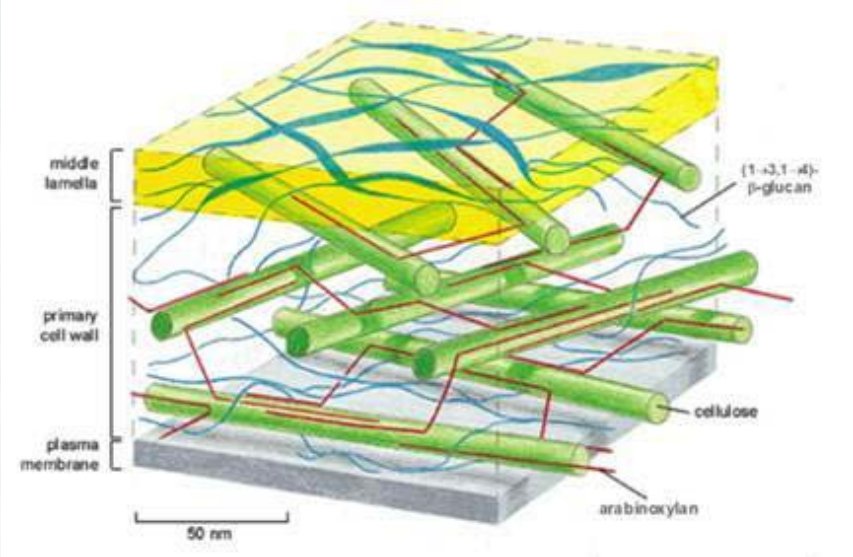
Loop engineering of a thermostable GH10 xylanase to improve low-temperature catalytic performance for better synergistic biomass-degrading abilities

Reporter：Shuai You

01 Background



Biomass

Pretreatment

- Acid or alkali
- Ammonia recovery infiltration (ARP)
- Ammonia fiber expansion (AFEX)
- Steam blasting

Xylanase + Cellulase

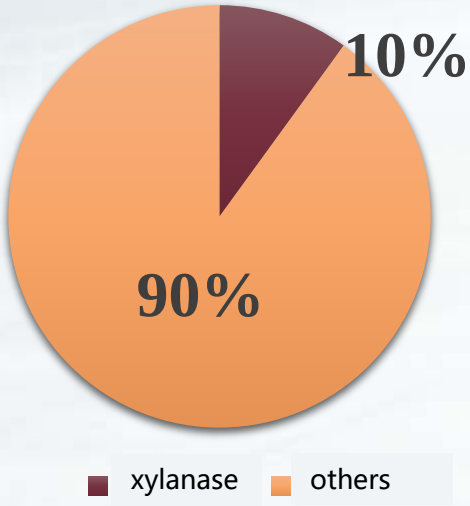
Improve efficiency →

Wide industrial applications

Song H T , Gao Y , Yang Y M , et al. Bioresource Technology, 2016, 219:710-715.

01 Background

Xylanase Ratio



- ✓ Improve digestibility
- ✓ Reduce the risk of gastrointestinal diseases

inefficient

Enzyme inactivation at high temperature granulation stage

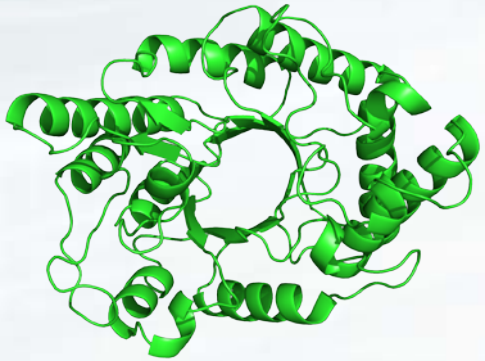
(70℃~80℃)

Low enzyme activity at body temperature

xylanase

Baker J T, Duarte M E, Holanda D M, et al. Animals, 2021, 11(3): 609.

01 Background

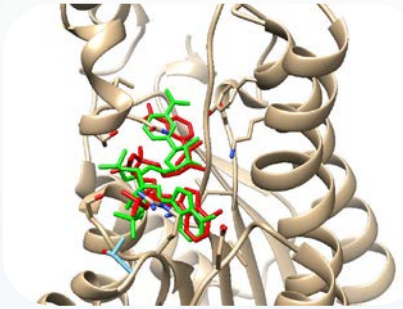


GH10 xylanase

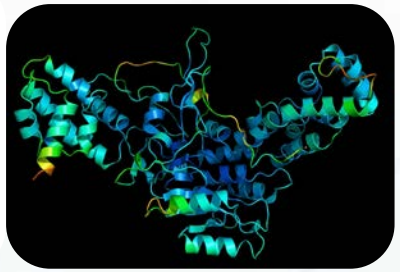
Molecular modified

The Loop closed to the active center plays an important role in enzyme catalysis.

Rational design



AutoDock



PyMOL

Clustal Omega、NCBI、

SWISS-MODEL et.al

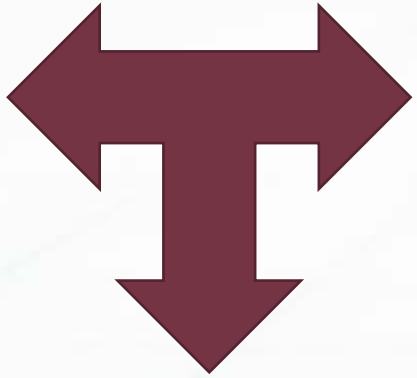
WT： XYL10C_ΔN

Liew K J, Ngooi C Y, Shamsir M S, et al. Protein Expression and Purification, 2019, 164:105464.

02

Aim

Efficient degradation of lignocellulose



Enhance enzyme catalytic activity at low temperature

Target xylanase

03 Method

Molecular docking and MD simulation → Identify the mutation site (43 sites) and the design of primers

04 Results

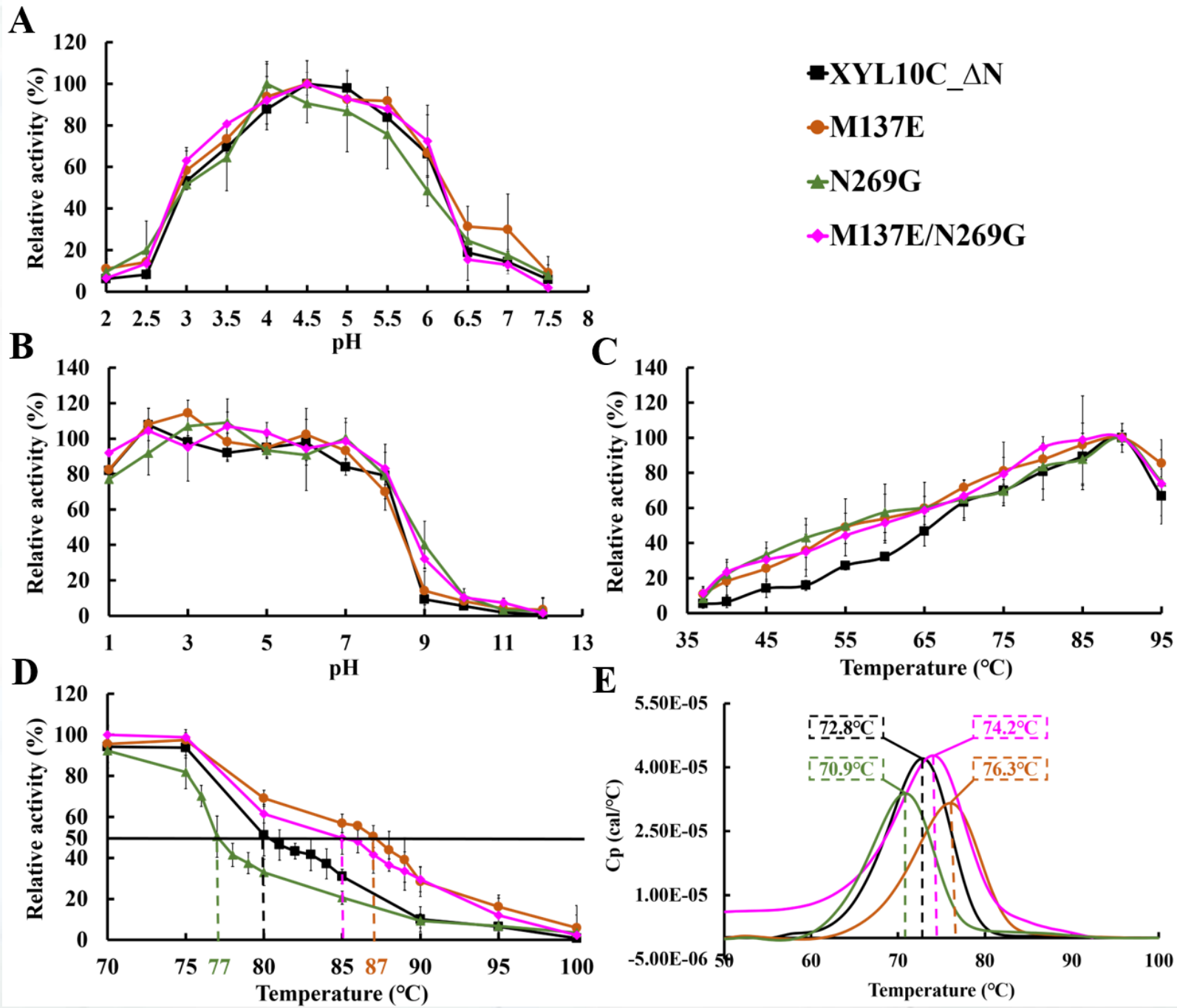


Fig 1. Enzymatic properties of the purified recombinant wild-type XYL10C_ΔN and its three mutants.

(A) The optimal pH of each enzyme .

(B) pH-activity profiles tested at the optimal temperature for each enzyme (90°C).

(C) pH stability profiles.

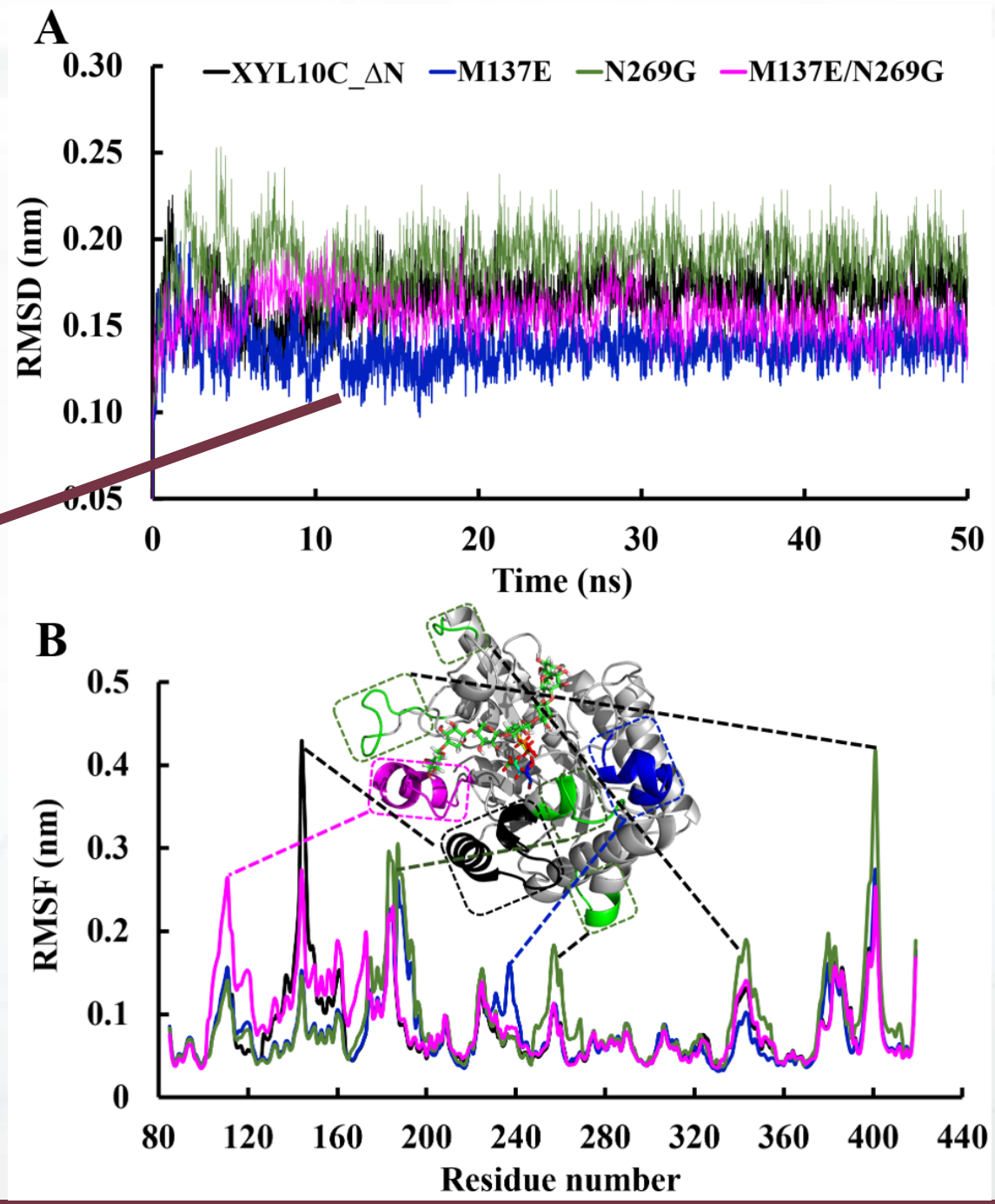
(D) Temperature-stability profiles (T_{50}).

(E) Thermograms determined by using DSC.

(F) Half-lives of wild-type XYL10C_ΔN and its mutants at 65 °C.

04 Results

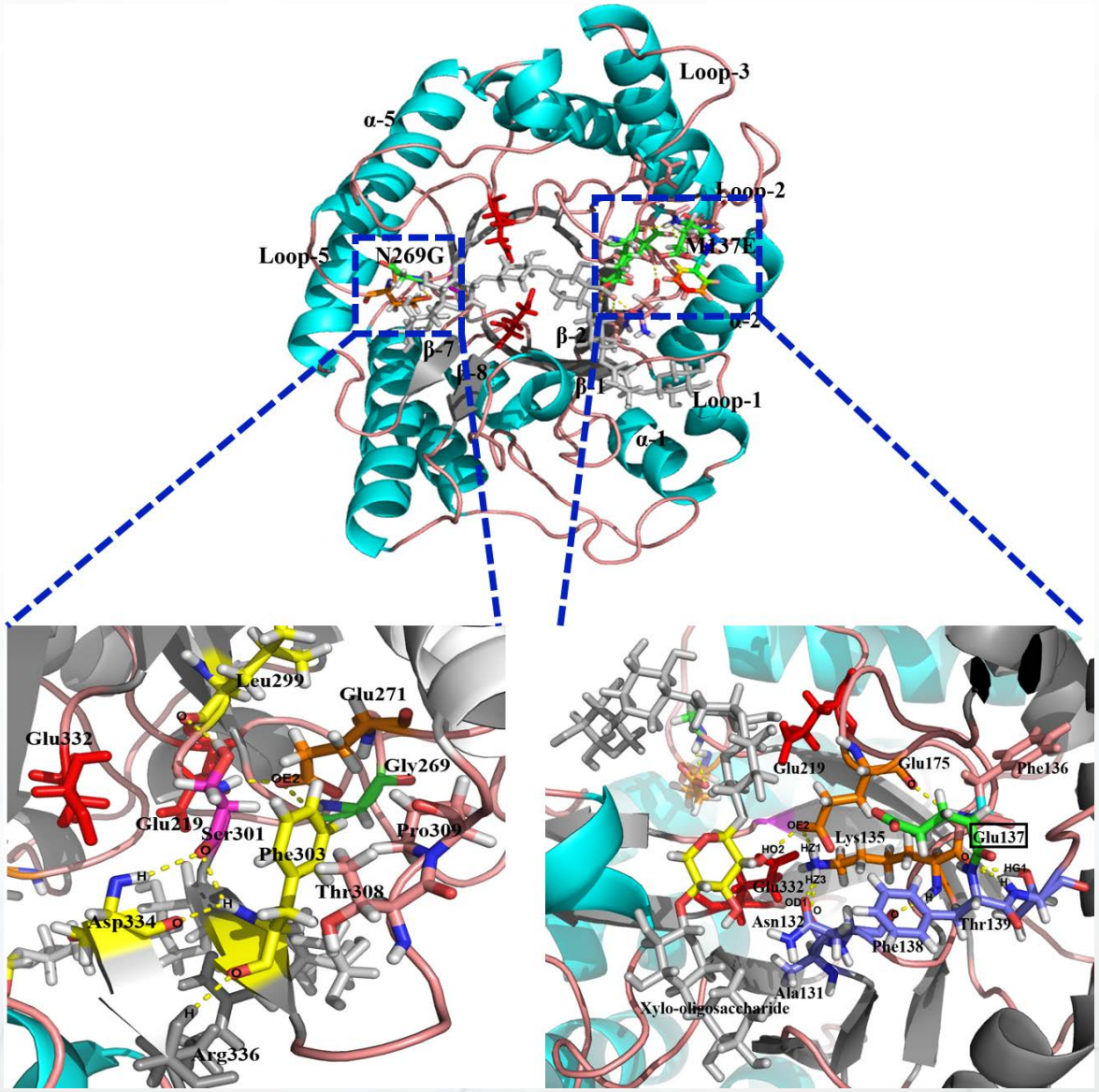
Thermostability:
M137E>M137/N269G>WT>N269G



04 Results

Stabilizes the conformation of the substrate binding channel

Enhances the interaction between the enzyme and the substrate molecule



04 Results

37℃

Substrate	Beechwood xylan				
Enzyme	K_m (mg/mL)	K_{cat} (s^{-1})	V_{max} (μmol/min·mg)	k_{cat}/k_m (mL/s·mg)	Specific activity (U/mg)
XYL10C_ΔN	1.39 ± 0.13	440 ± 23	700 ± 36	320 ± 17	510 ± 33
M137E	0.99 ± 0.03	800 ± 7	1260 ± 11	802 ± 21	1200 ± 89
N269G	1.02 ± 0.12	600 ± 12	950 ± 20	590 ± 59	990 ± 67
M137E/N269G	0.85 ± 0.06	1130 ± 12	1770 ± 18	1330 ± 78	1610 ± 91

04 Results

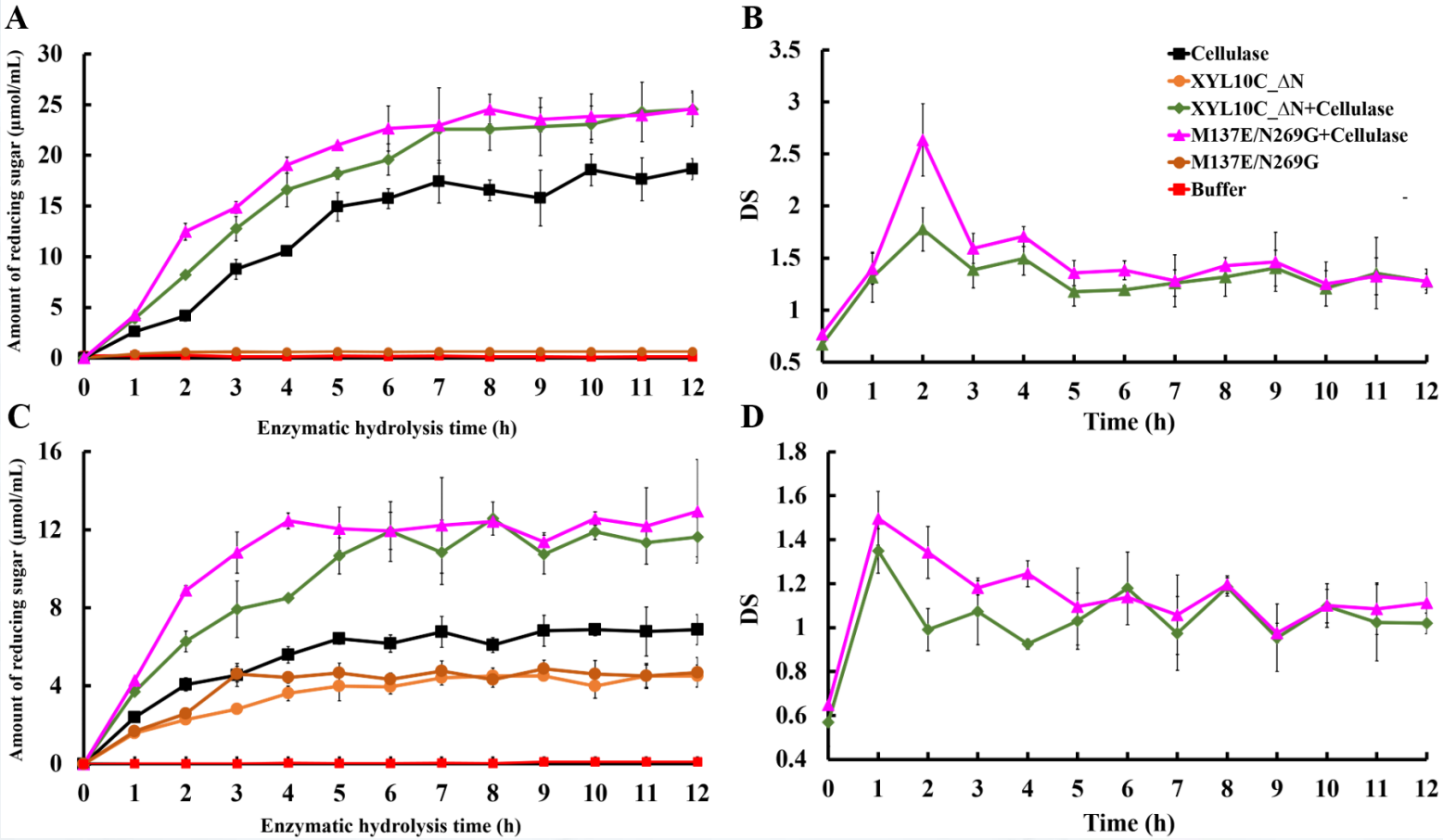


Fig 2. Time-course hydrolysis of mulberry bark and corn cob. Separate hydrolysis: 5 U each of cellulase or xylanase simultaneous hydrolysis: 50 U each of cellulase and xylanase; control: no enzyme added (square) to substrates for 12 h (A) Mulberry bark (C) Corn cob. The DS curve for the mulberry bark samples are shown. Enzyme loading: cellulase 50 U, xylanase 50 U. The changes in the dry weight of the mulberry bark during separate and simultaneous hydrolysis with cellulases and xylanase after 24 h are shown (B) Mulberry Bark (D) Corn cob.

04 Results

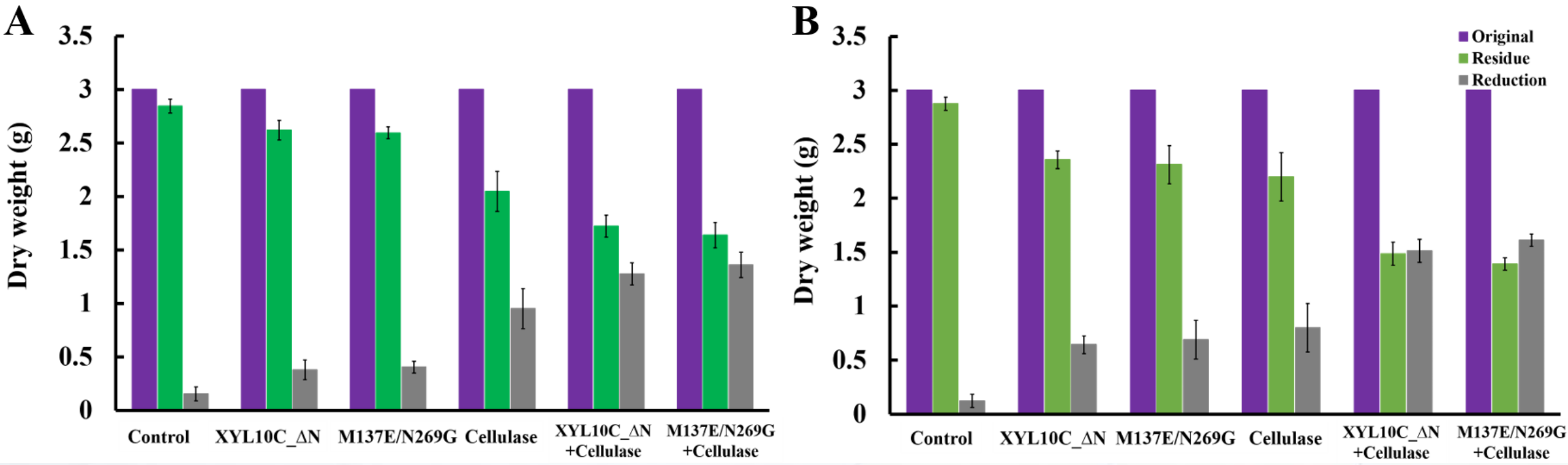


Fig. 3. Change in dry weight in three lignocellulosic substrates during separate and simultaneous hydrolysis with cellulases and xylanase after 72 h. (A) corncob . (B) mulberry. (C) wheat bran. (A) Mulberry bark (B) Corn cob

04 Results

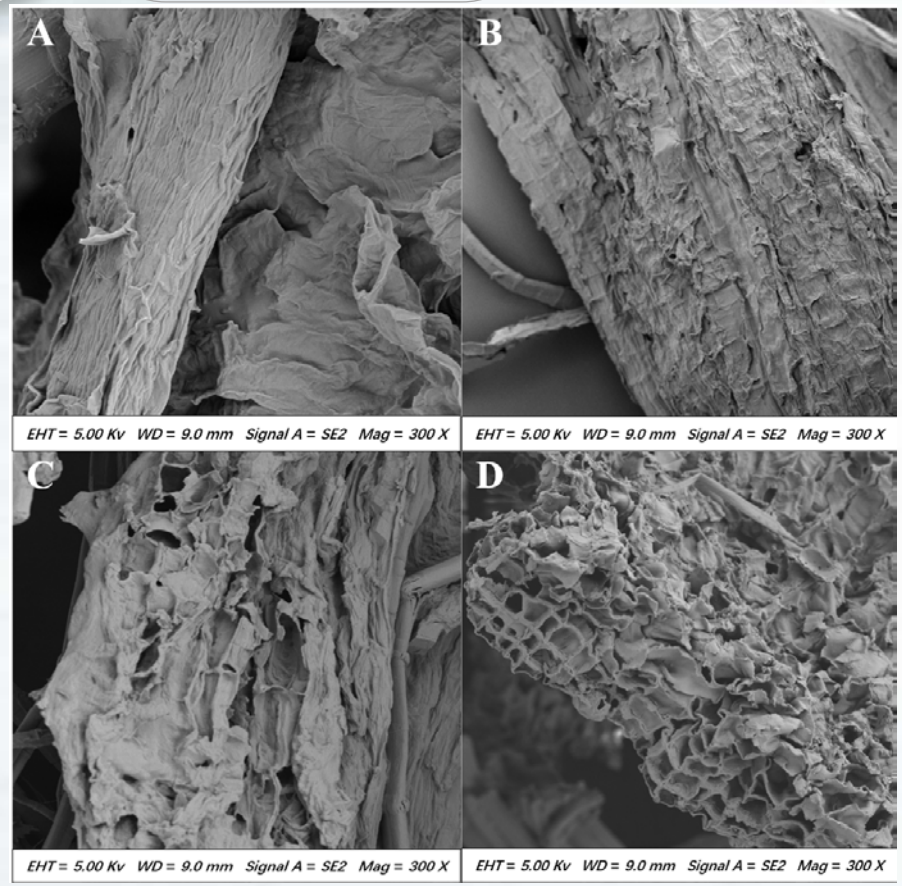


Fig. 4. Electron microscopy images of the micro structure of the mulberry bark samples treated with different enzymes. (A) Buffer treatment for 24 h (B) M137E/N269G alone (C) Cellulase alone (D) mixture of M137E/N269G and cellulase

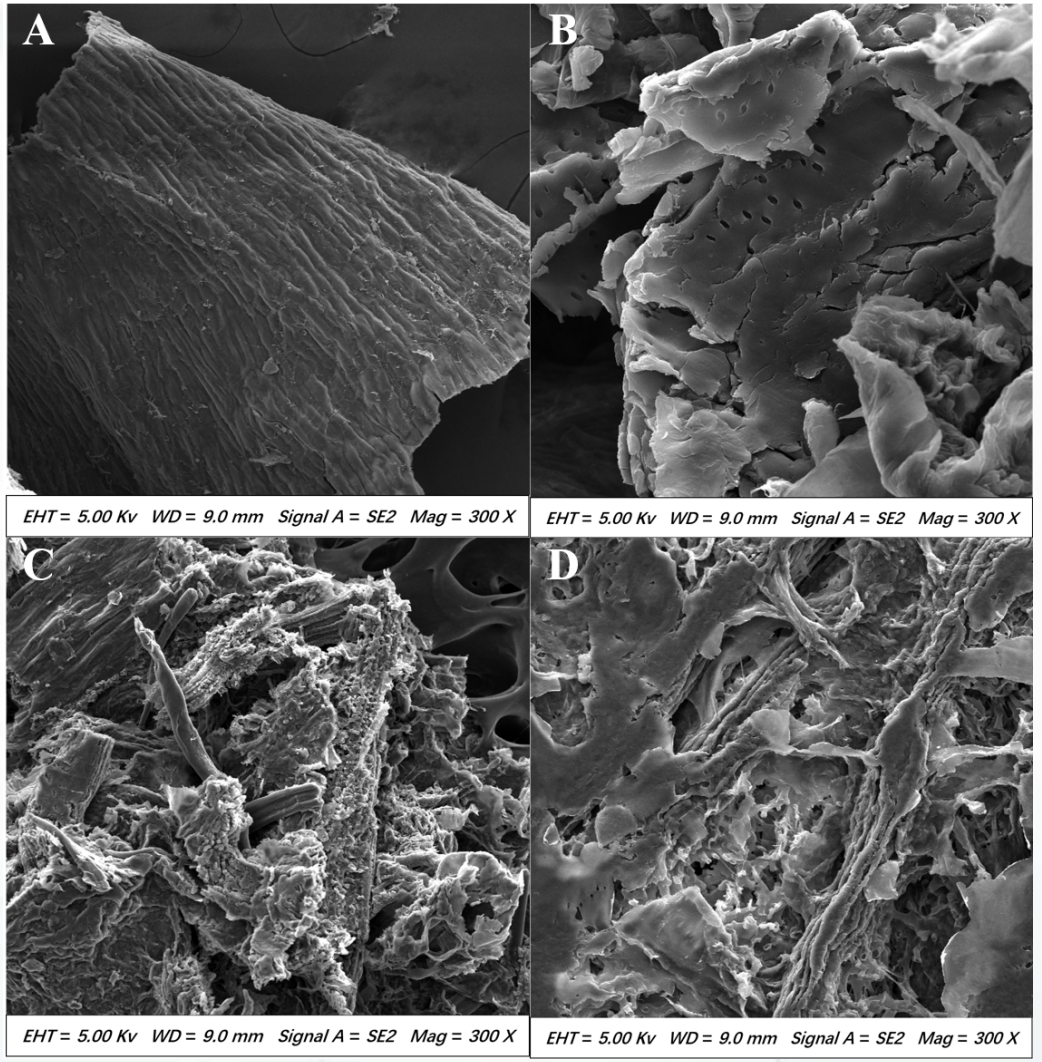


Fig. 5. Electron microscopy images of the micro structure of the corn cob samples treated with different enzymes. (A) Buffer treatment for 24 h (B) M137E/N269G alone (C) Cellulase alone (D) mixture of M137E/N269G and cellulase.

04 Results

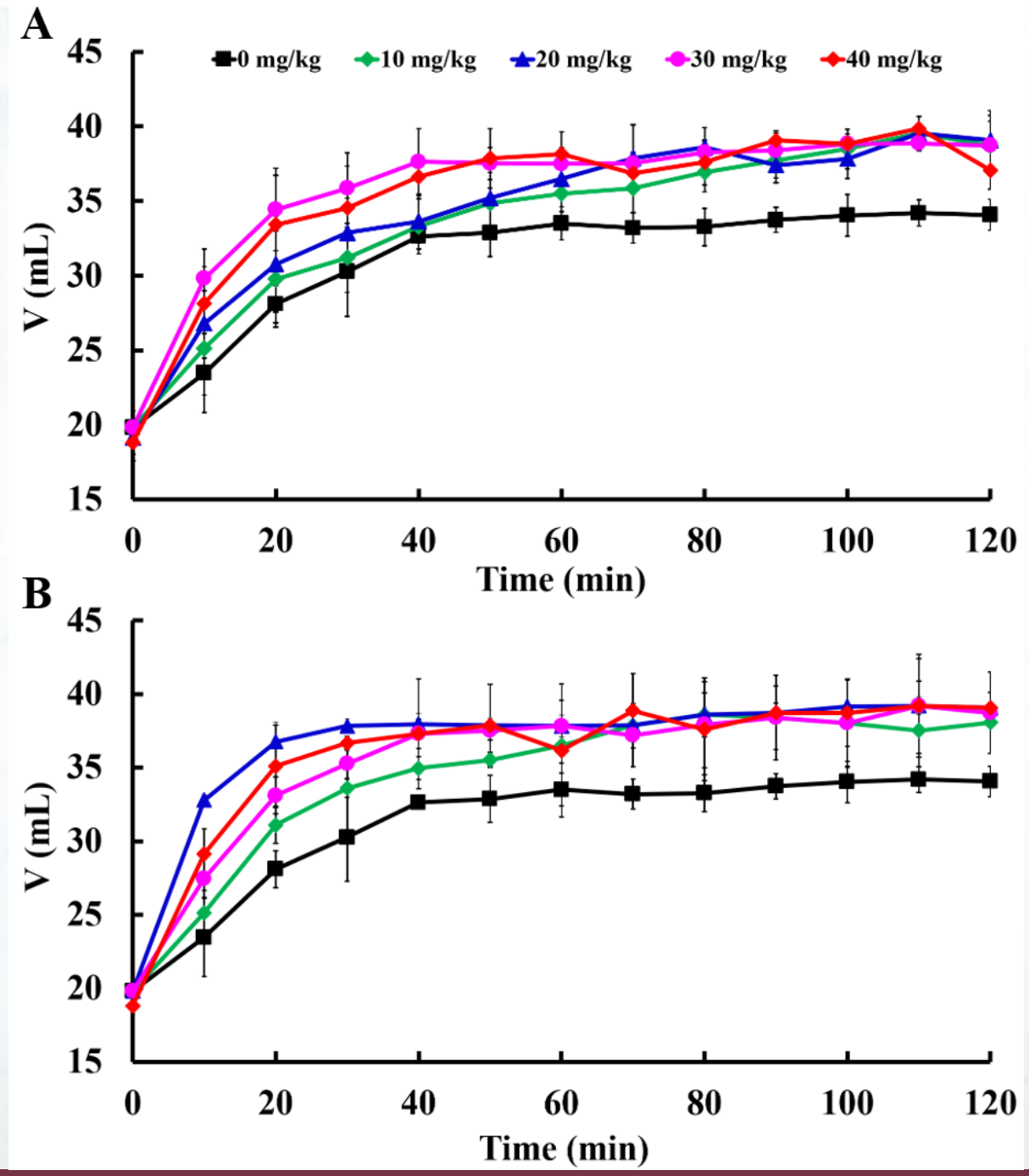


Fig. 6. The effect of xylanases in different additions on the volume of bread. (A) The wild-type XYL10C_ΔN with different additions. (B) The mutant M137E/N269G with different additions.

04 Results

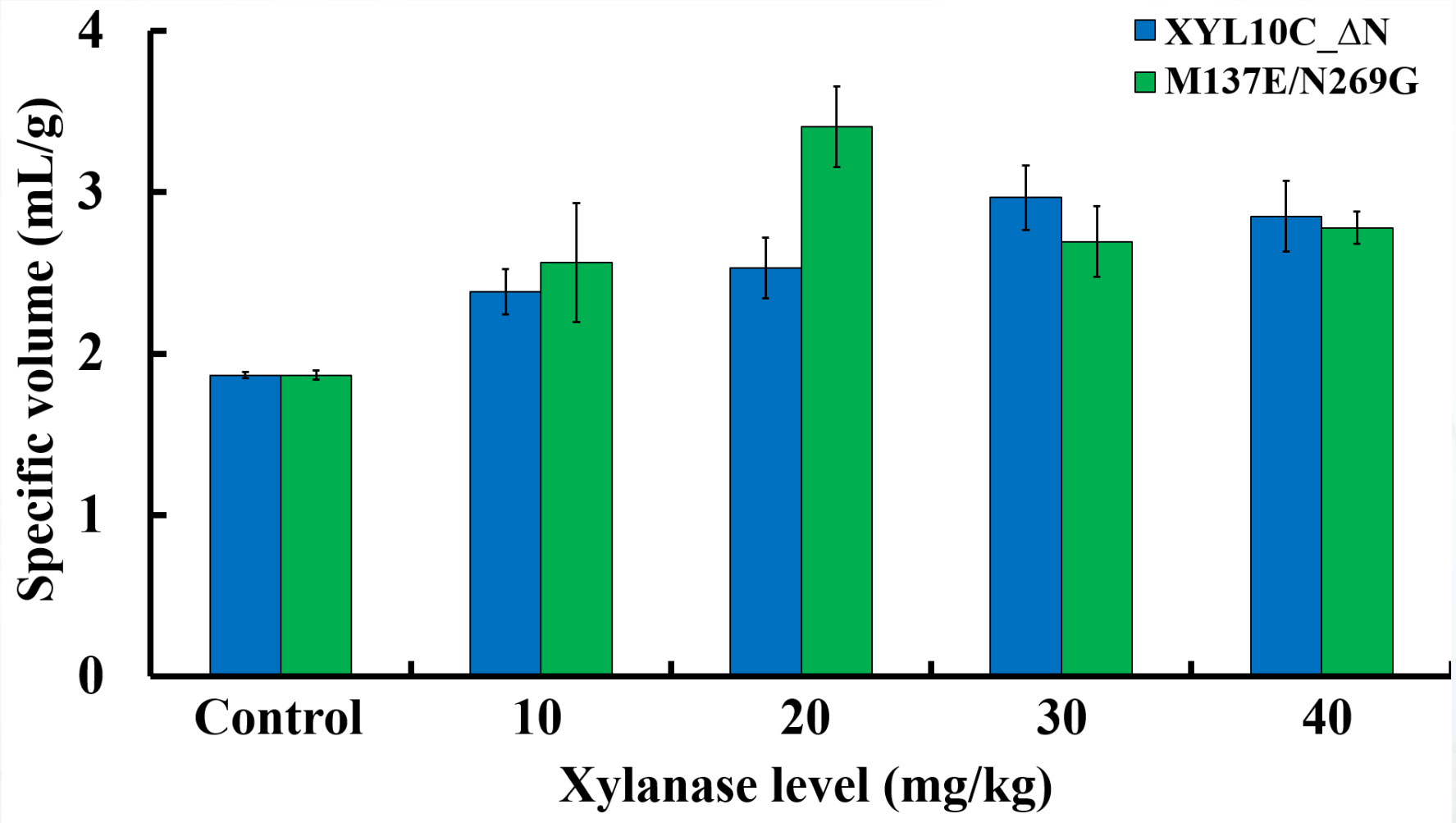


Fig. 7. The effect of xylanase level in different additions on the specific volume of bread. (A) The wild-type XYL10C_ Δ N with different additions. (B) The mutant M137E/N269G with different additions.

05 Conclusion

1. All the enzyme maintained similar pH and temperature optimal but the mutants showed notably improved catalytic performance under low temperature.

2. Loop region plays a vital role in hydrolysis of substrates, providing a reference for thermostable GH 10 xylanases engineering in improving their biochemical characteristics.

3. The successful improvement of XYL10C_ΔN makes the mutant produced in this study a potential xylanase for industrial applications on the degradation of lignocellulosic substrates even in diverse conditions.

Thanks for listening!

Reporter：Shuai You

Data：2021.06.17