PRODUCTION, CHARACTERIZATION AND PURIFICATION OF XYLANASE FROM *Staphylococcus aureus* MBXi-K4

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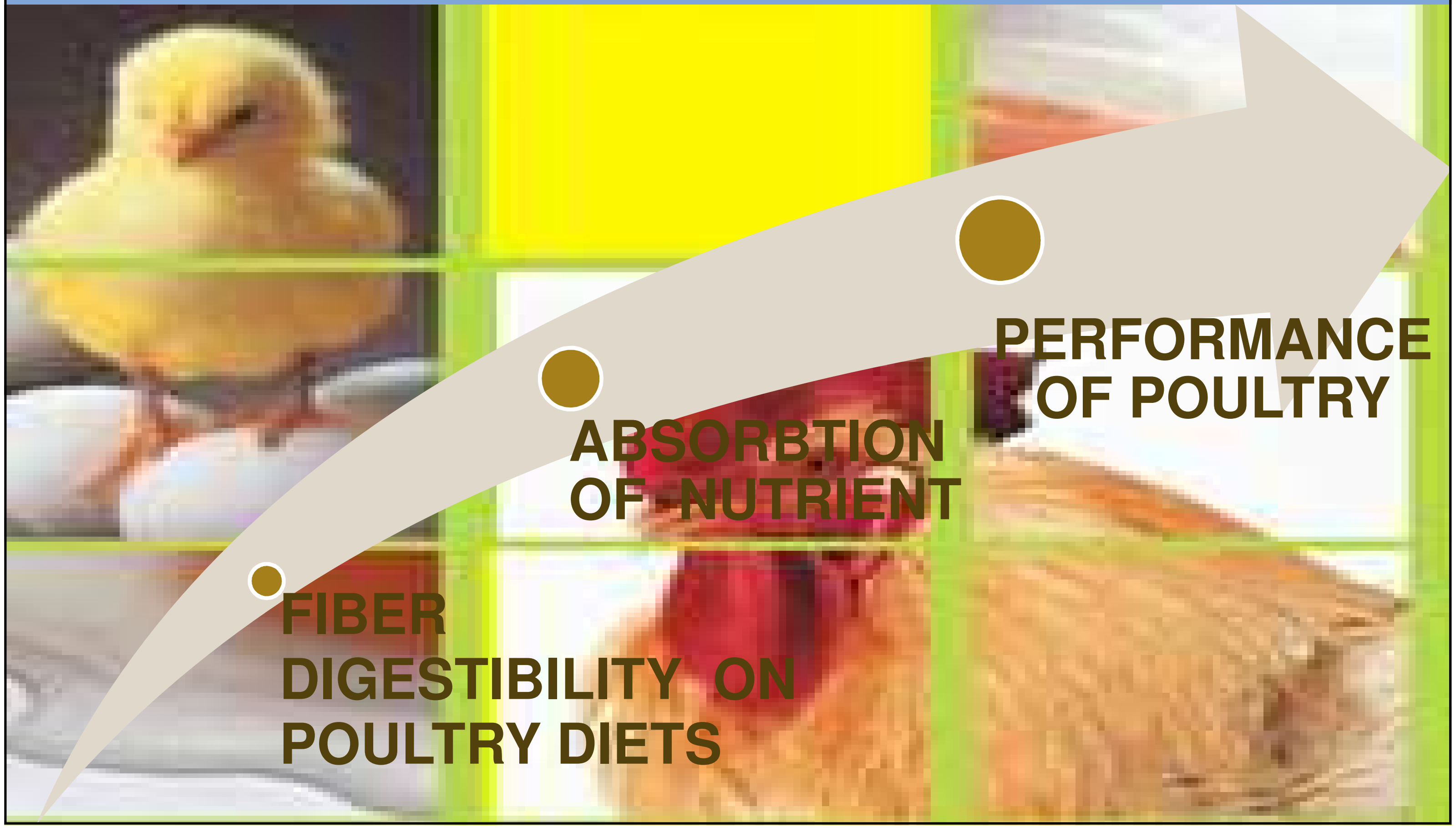
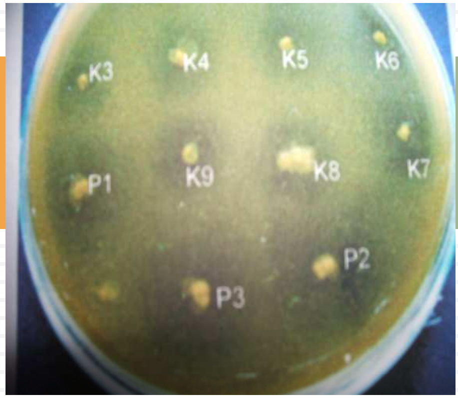
INTRODUCTION





Isolation and characterization of microbes (Inda,S 2006)

Xylanase production

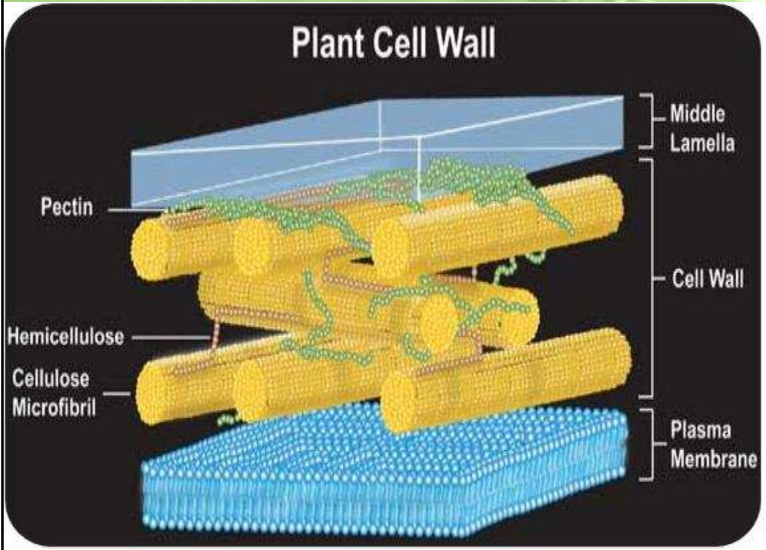
Characterizati on and purification of xylanase

Aims

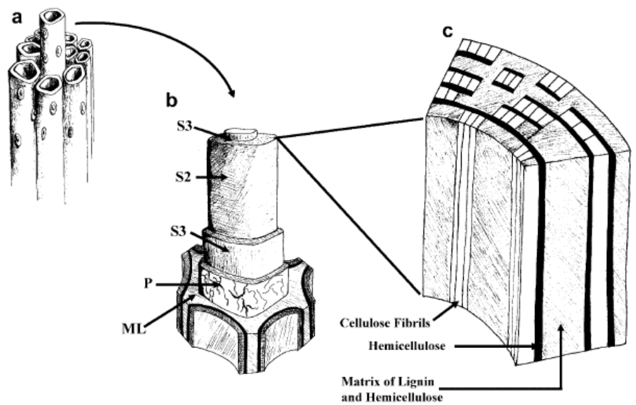
The objectives of this research are

- to produce xylanase in batch system bioreactor
- to characterize and purify xylanase from *Staphylococcus aureus* MBXi-K4 in order to explore its possibility as feed additive in pelleting poultry feed.

HEMICELLULOSE



- The second most abundance component of plant cell wall after cellulose.

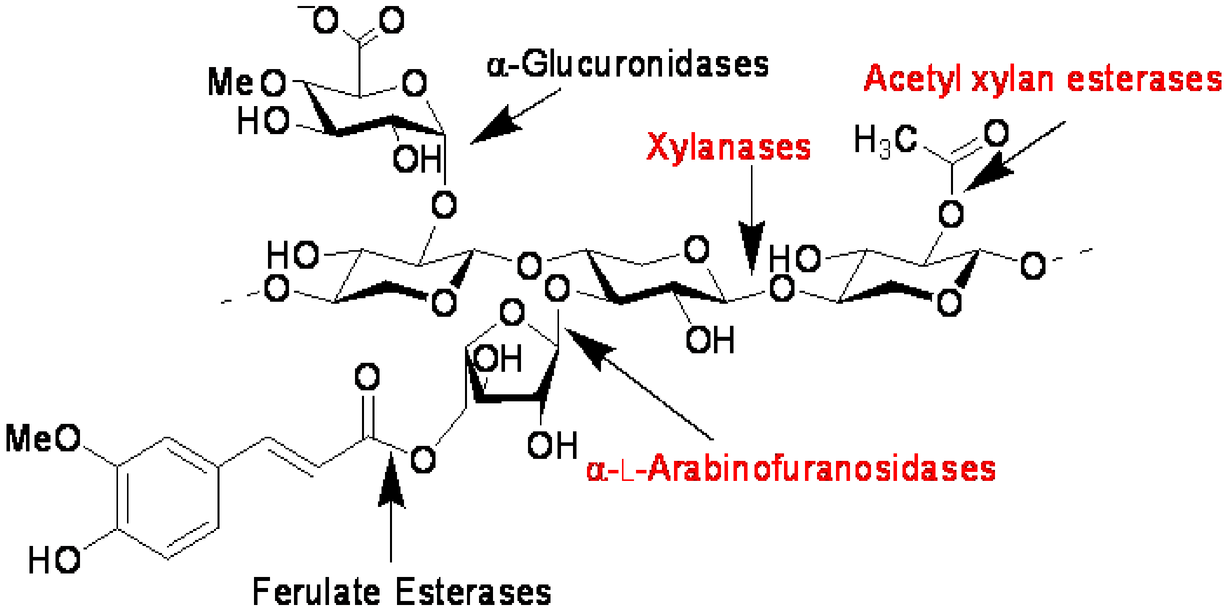


Two main composition of hemicellulose are hetero - 1,4-D- xylan and hetero-1,4-D-mannan

Perez et al. 2002

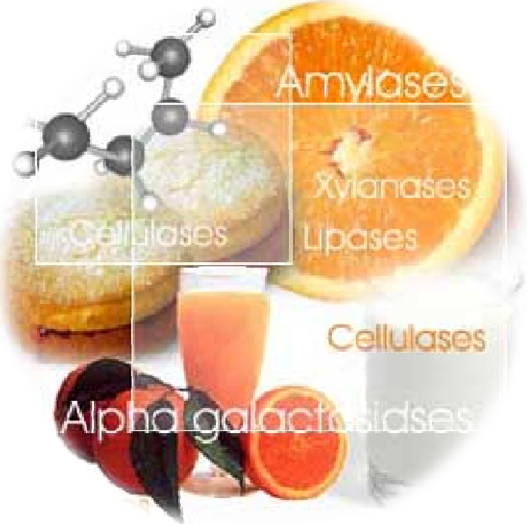
XYLANASE (EC 3.2.1.8)

Xylanase hydrolyze hemicellulose to its simple sugars (xylose and xylooligosaccharides)



Xylanase aplication on Industry

- Production and recycling of paper
- Delignification of pulp
- Feed Industry
- Food and beverage Industry
- Textile Industriy
- Biopharmaceutical Production



METHODS

Screening of bacteria that produce xylanase thermostable

Production of Xylanase

Xylanase production on 2 L bioreactor (STR) with process condition: aeration1 vvm, agitation 160 rpm,

Growth temperature: 37oC, pH: 7

Analysis of Process Kinetics : (μ, Yx/s, Yp/s)

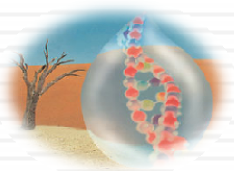
Analysis of Enzymatic Kinetic : (Km dan Vmaks)

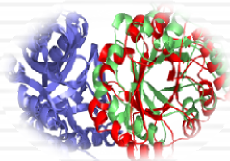
Enzyme Characterization :

- pH and thermal stability
- Profil of SDS PAGE and zymogram

Purification of Enzyme

- Ammonium sulfat Precipitation
- Dialysis
- Gel Filtration Chromatography





XYLANASE PRODUCTION

1 Peremajaan isolat

2 propagation

3 inokulation

4 Fermentation

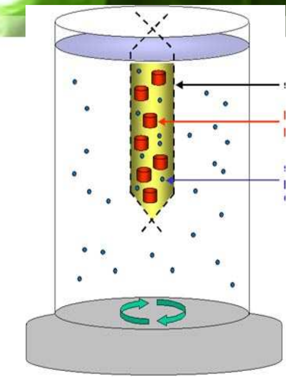
Substrate	**Komposisi (% b/v)**
Yeast ekstract	0.2
K_2HPO_4	1.5
$Mg.SO_4.7H_2O$	0.025
Oat Spelt xylan	0.7
NaCl	0.25
NH_4Cl	0.5
Na_2HPO_4	0.5
pH	7.0

Temperature: 37oC
pH : 7
Aeration: 1 vvm
Agitation: 160 rpm
Substrate: pollard xylan

ENZYME PURIFICATION

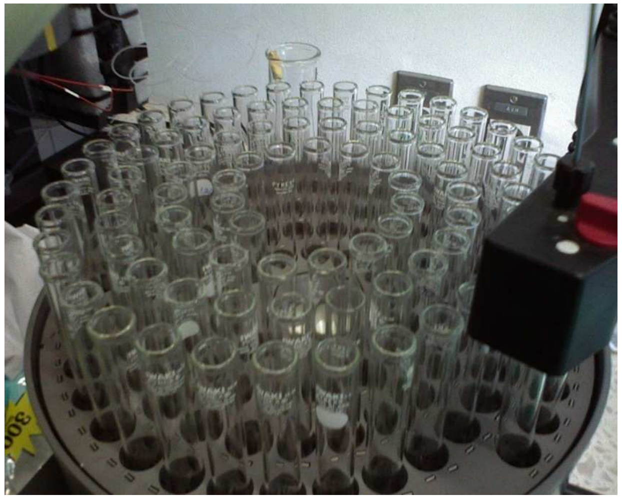
Ammonium Sulphate precipitation

Ammonium sulphate concentration 40% - 60%



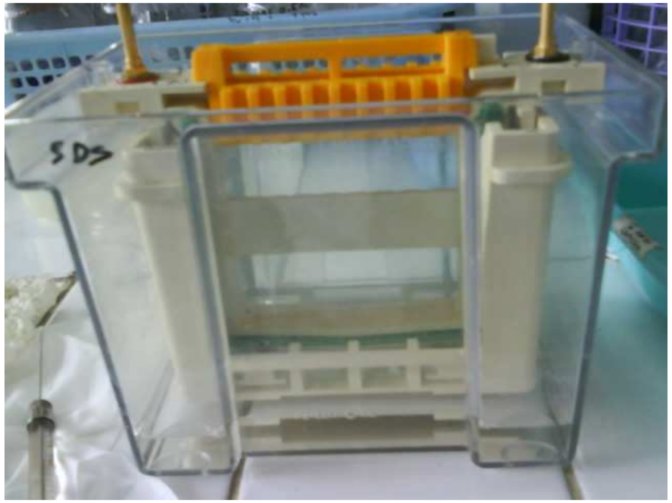
Dialysis

Membran Dialysis, MWCO 12kDa



Gel filtration chromatography
Matrix: Sephadex G-100

SDS-PAGE dan ZYMOGRAM



SDS-PAGE

100 volt, 50 mA for 2 hours
Silver staining

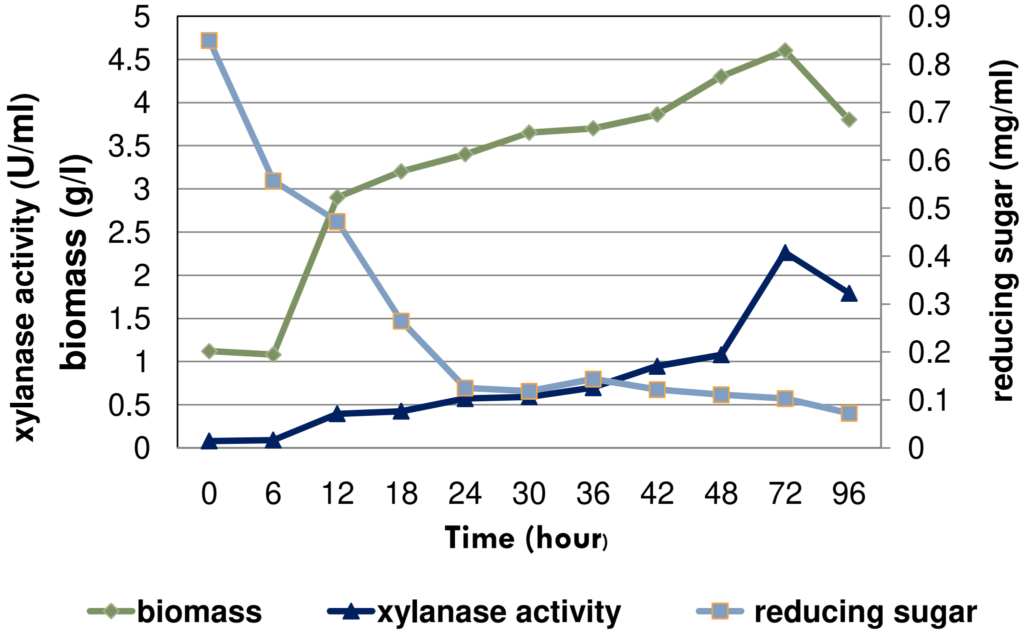
Substrat Oatspelt Xilan 0,7 %
Staining : Congo Red

Zymogram

RESULTS

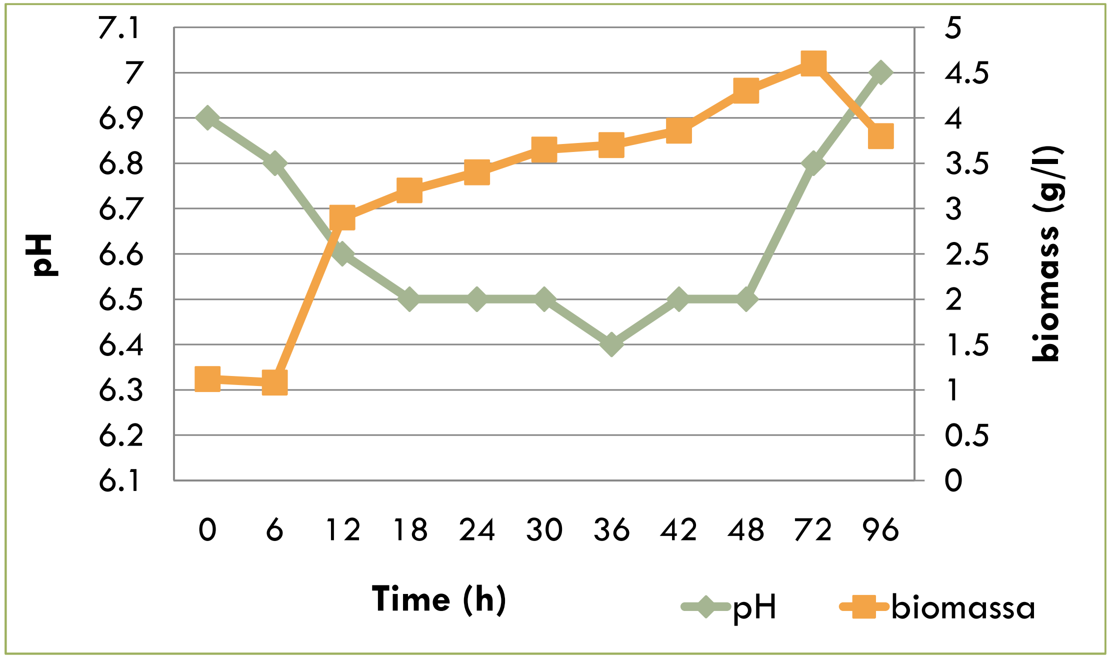
1\. Kinetika Pertumbuhan Mikroba dan Produksi Enzim

Biomass Production



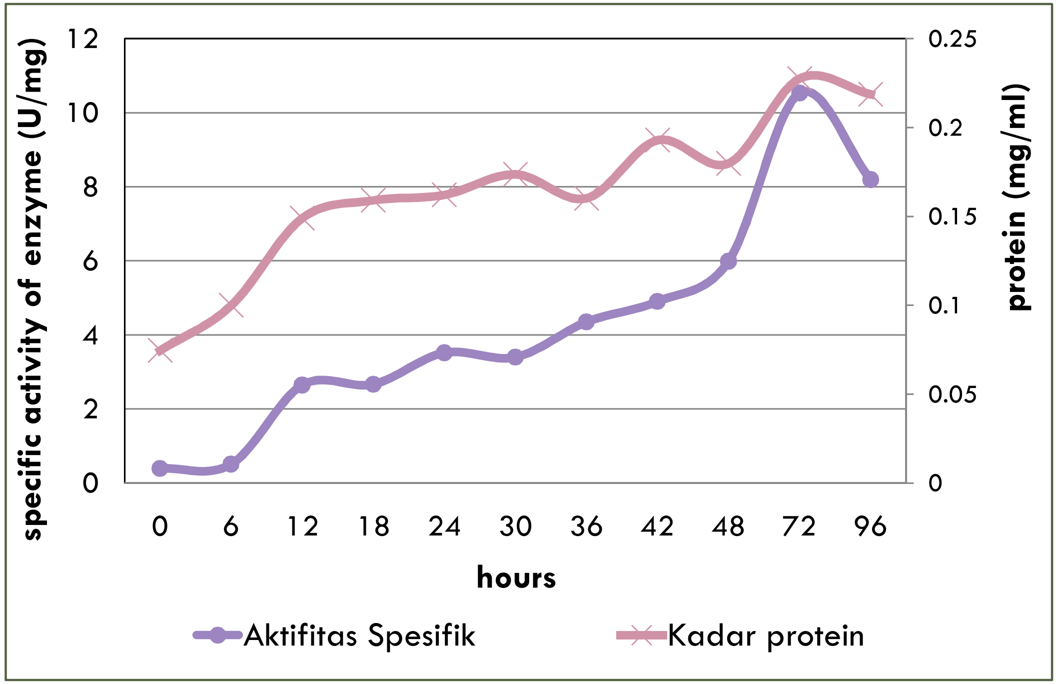
- Maximum biomass prodution of 4,6 (g/l) → at 72 hours
- Maximum xylanase activity of 2,26 (U/ml) → at 72 h of fermentation
- Growth associated xylanase production

pH



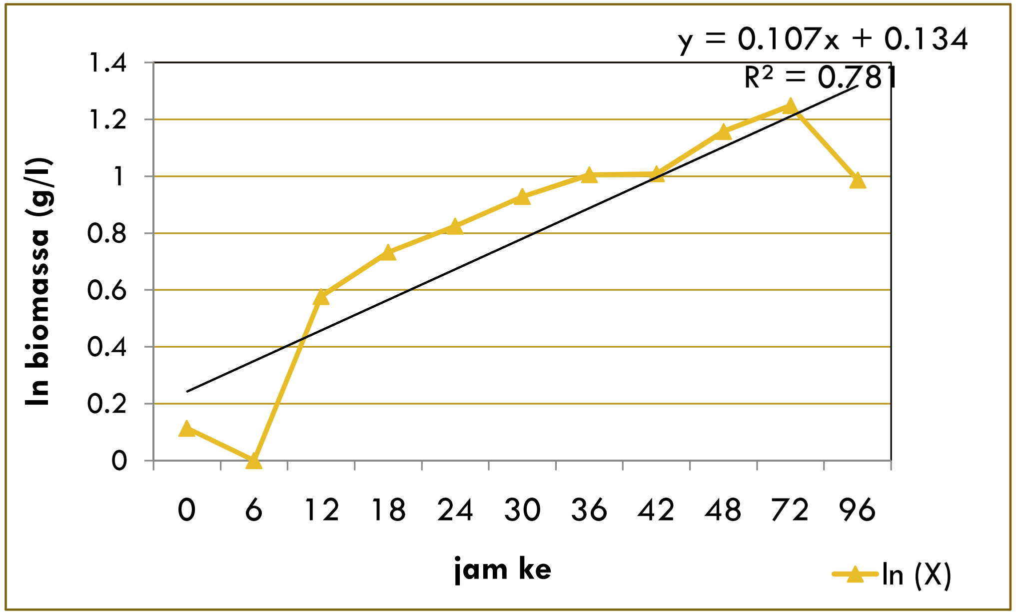
- Decrease of pH value in related to biomass production

Enzyme Production



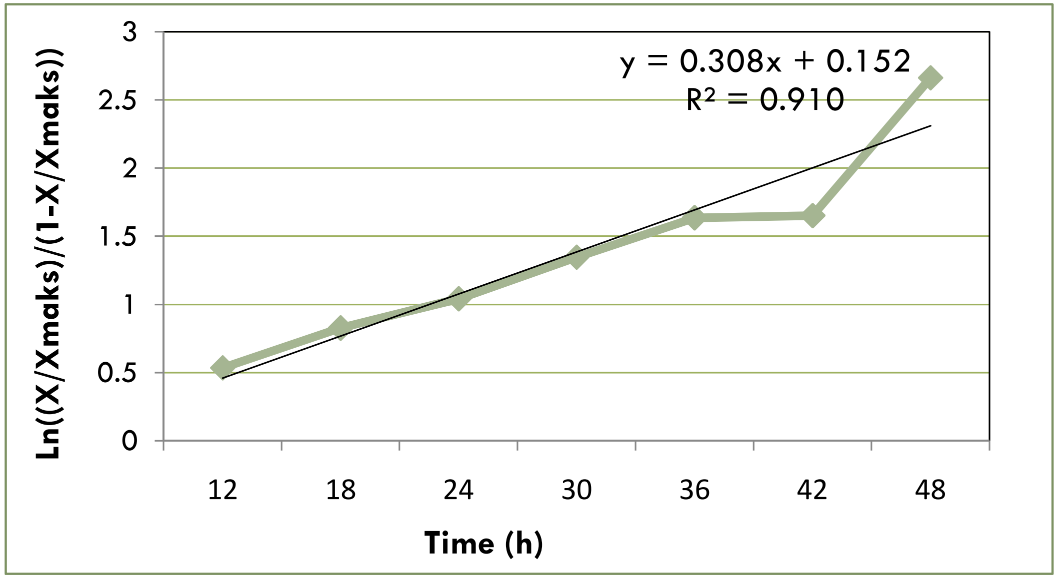
- Xylanase production had a same trend with consentration of protein.
- Xylanase is a primer metabolite product for *S.aureus*
- Maximum production of xylanase was at 72 of fermentation = 10,5 (U/mg)

Kinetics of Cultivation *S.aureus* MBXi-K4



- $\ln (Xt/Xo) = \mu \Delta t$
- μ (Spesific Growth of Biomass) = 0,107 /jam

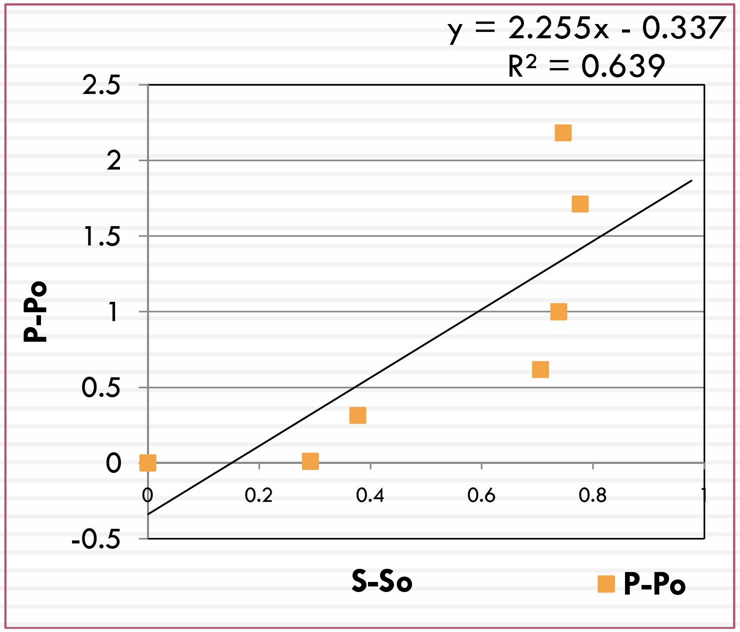
Maximum of Spesific Growth Rate of Biomassa (μ max)



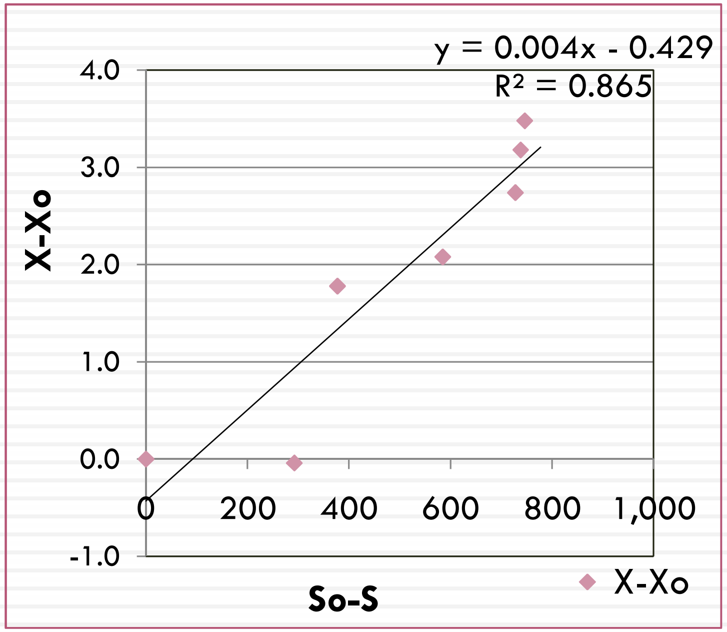
- μ max = 0,308 /hour
- μ max is costant along the fermentation

Yield of Biomass (Yx/s) and Product (Yp/s)

Yield of Product (Yp/s)



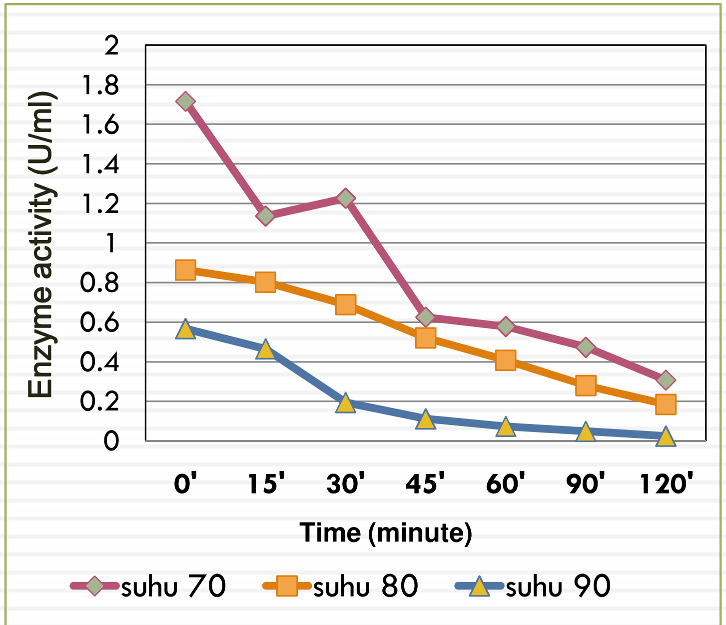
Yield of Biomass (Yx/s)



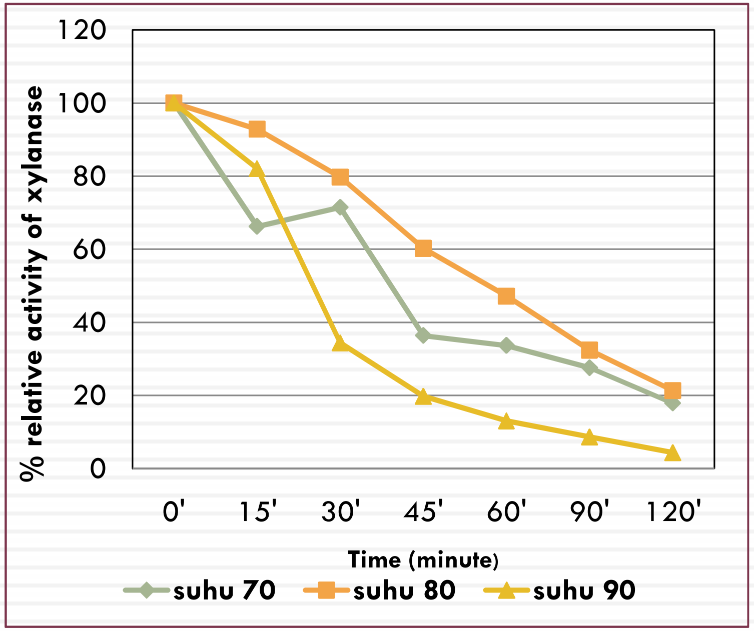
- $Y_{p/s}$ = 2,25 (U enzyme /mg substrate)
- $Y_{x/s}$ = 0,004 g biomass/g substrat

2. CHARACTERIZATION OF XYLANASE

Enzyme stability on temperature



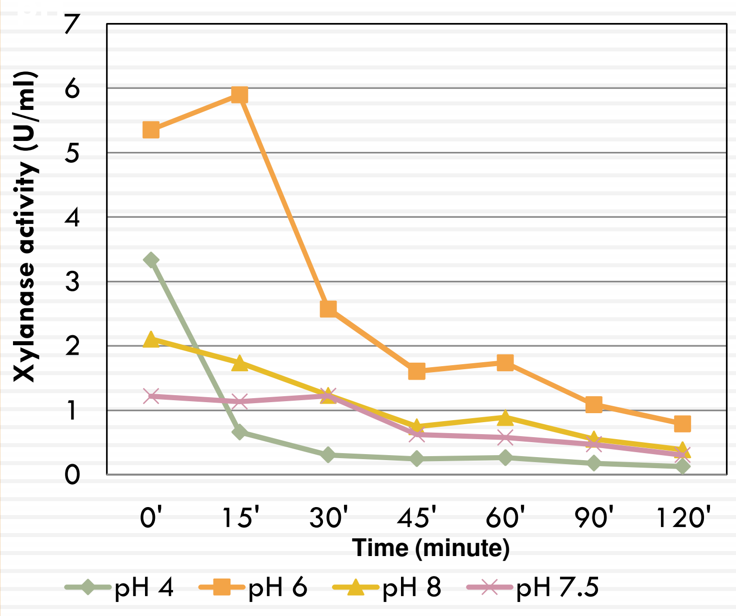
Relative activity on temperature



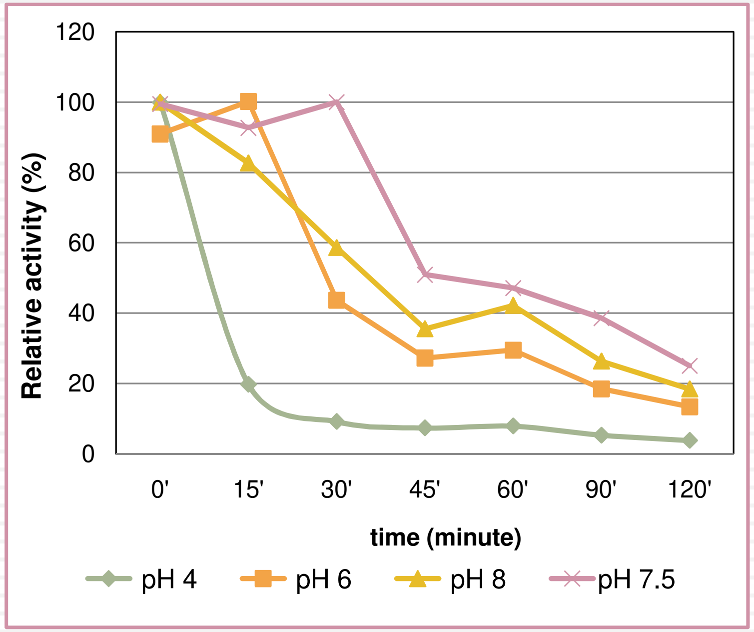
- Xylanase activity decrease in related to increase of temperature

Stability of Enzyme on pH

Enzyme stability on pH

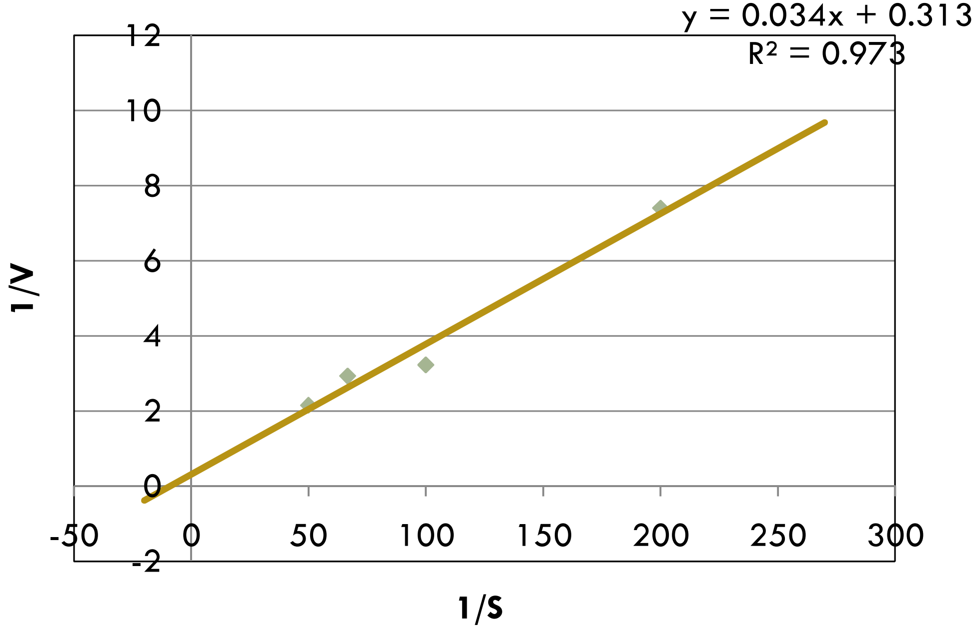


Relative activity of enzyme on pH



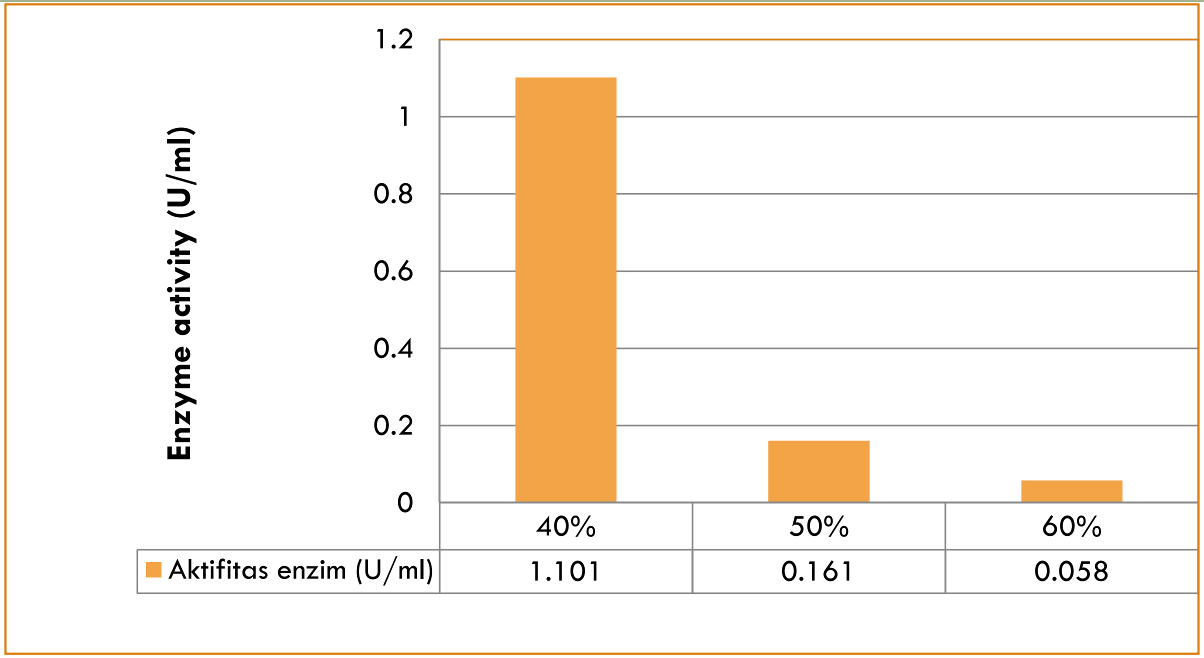
- Maximum activity of xylanase of pH 6 (phosphate buffer)
- Xylanase more stable on pH 7,5

KINETICS OF ENZYMATIC REACTION



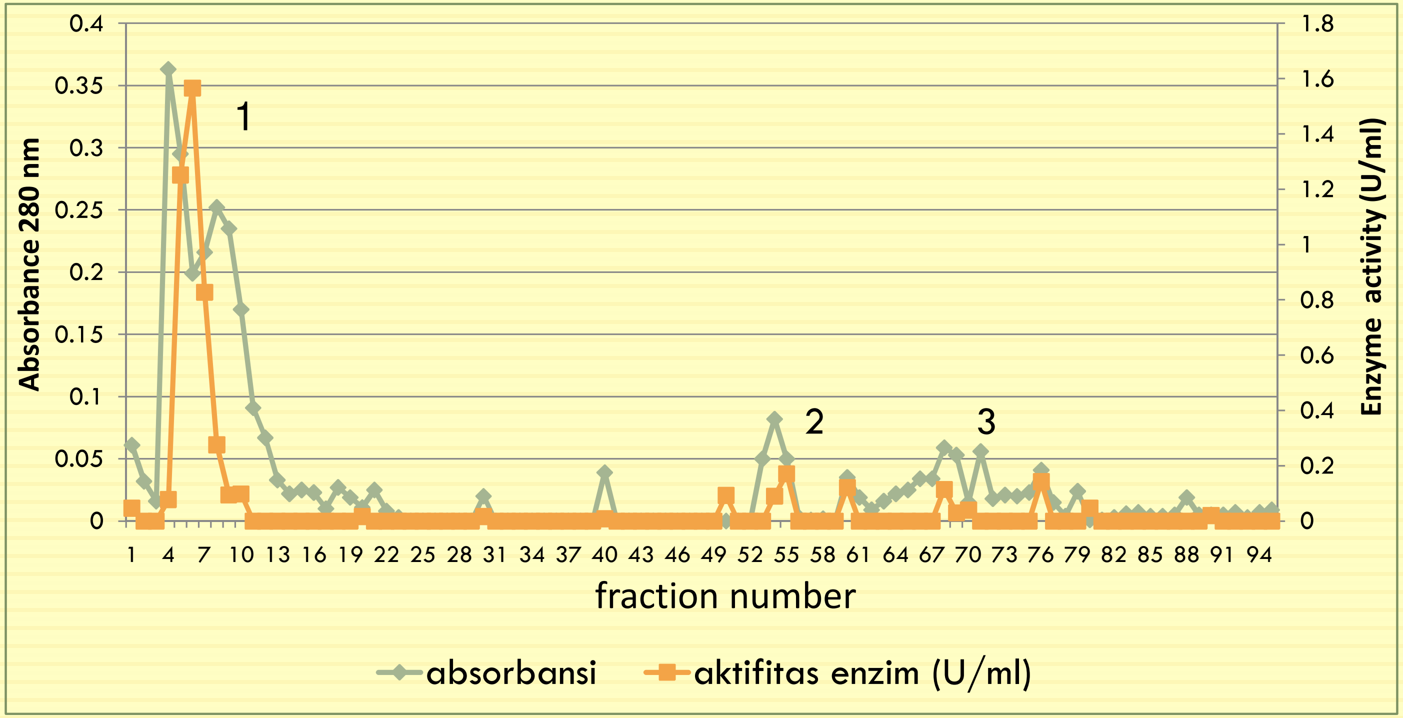
- Vmaks = 3,195 (µmol xilosa/min.ml)
- Km = 1,086 (mg/ml).
- Oatspelt Xylan concentration : 0,5% - 2%

PURIFICATION OF XYLANASE



- Purification of xylanase use Ammonium Sulphate precipitation (40% - 60%)
- Best concentration of ammonium sulphate : 40%

GEL FILTRATION CHROMATOGRAPHY

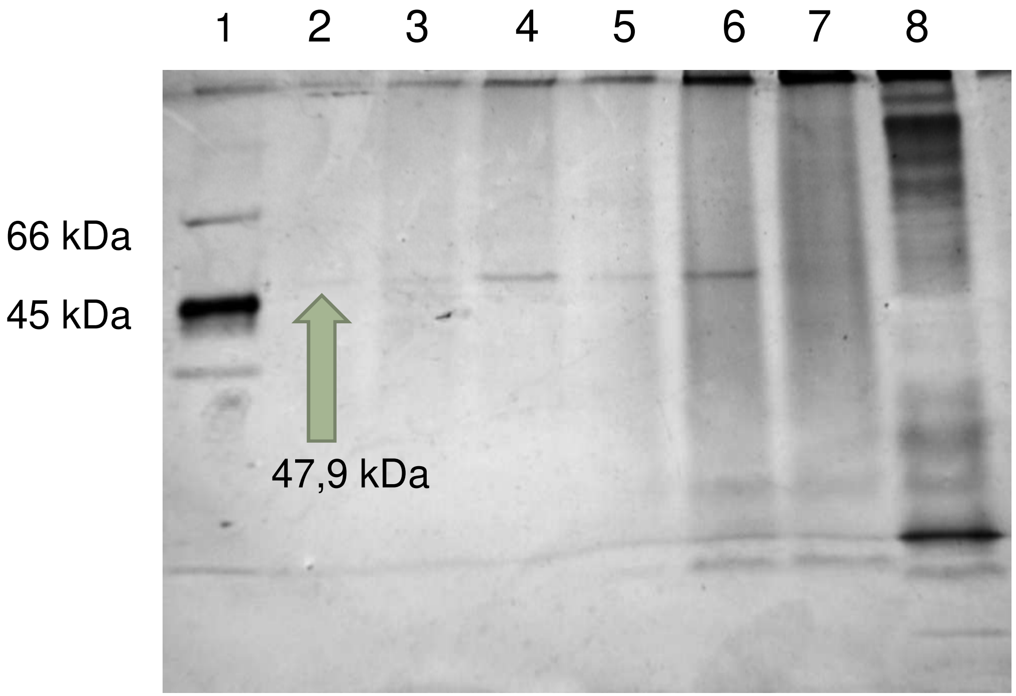


- Terdapat 3 puncak protein (fraksi nomer 4-6, 54-55 dan 68-69)
- Aktifitas spesifik xilanase tertinggi pada fraksi no 6= 383,9 U/mg
- Sebagian besar protein yang diperoleh merupakan enzim target (xilanase)

Purification Steps

Step	Volume (ml)	Total Protein (mg)	Total Activity (U)	Spesific Activity (U/mg protein)	Recovery (%)	Fold
Crude enzyme	81	3.32	109.01	32.82	100	1
Amm.sulfat precipitation	10	0.48	18.06	37.39	16.57	1.14
Sephadex G-100	3	0.012	4.69	383.90	4.31	11.69

SDS - PAGE



well number

1. marker LMW Pharmacia
2. Gel Filtration. fraksi no 6
3. fraksi no 5
4. fraksi no 4
5. diálysis
6. Ammonium sulphate precipitation
7. Ammonium sulphate precipitation no 2
8. Crude ekstract

ZYMOGRAM PROFILE

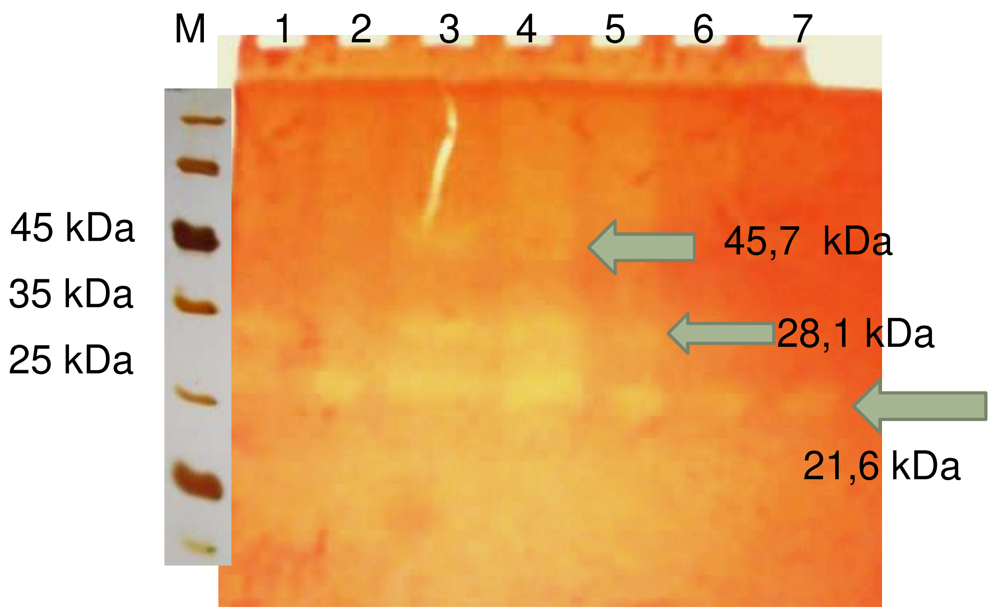


Figure 6. Zimogram profile of purified xylanase from *S.aureus* MBXiK4, stained with Congo Red (1) LMW standards; (2) crude enzyme,(3) ammonium sulfat precipitation,(4) freeze dried crude enzyme ,(5)freeze dried ammonium sulfat precipitation ,(6) xylanase after dialysis , (7)fraksi no 4 – 6 of purified xylanase on Sephadex G-100, (8) fraksi no 6 of purified xylanase on Sephadex G-100.

CONCLUSIONS

- Gel Filtration Chromatography technique can purify xylanase 11.69 times of crude extract enzyme.
- Kinetic of enzymatic reaction of Km : 1.086 (mg/ml) and Vmax : 3.195 (µmol xilosa/min/ml)
- *S.aureus* MBXiK4 had 3 kinds of xylanase with MW of 45.6 kDa, 28.1 kDa and 21.63 kDa
- Xylanases from S.aureus MBXiK4 are moderate thermostable enzyme which had relative activity more than 70% of its activity at 70oC for 30 minutes and active on pH range 4 – 8 with max activity at pH 6.
- Xylanase from *S.aureus* MBXiK4 may has an application on feed industry

THANK YOU

- Due-Like batch III