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Njoku, Stephen Ikechukwu; Jurado, Esperanza; Malmgren-Hansen, Bjørn; Kragelund, Caroline; Ruiz, Begoña; Uellendahl, Hinrich

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# Testing the effect of different enzyme blends on increasing the biogas yield of straw and digested manure fibers



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S.I. Njoku<sup>1</sup>, E. Jurado<sup>1</sup>, B. Malmgren-Hansen<sup>2</sup>, C. Kragelund<sup>2</sup>, B. Ruiz<sup>3</sup> and Hinrich Uellendahl<sup>1\*</sup>

<sup>1</sup> Section for Sustainable Biotechnology, Aalborg University Copenhagen, A.C. Meyers Vænge 15, DK-2450 Copenhagen, Denmark

<sup>2</sup> Danish Technological Institute, Kongsvang Allé 29, DK-8000 Aarhus C, Denmark

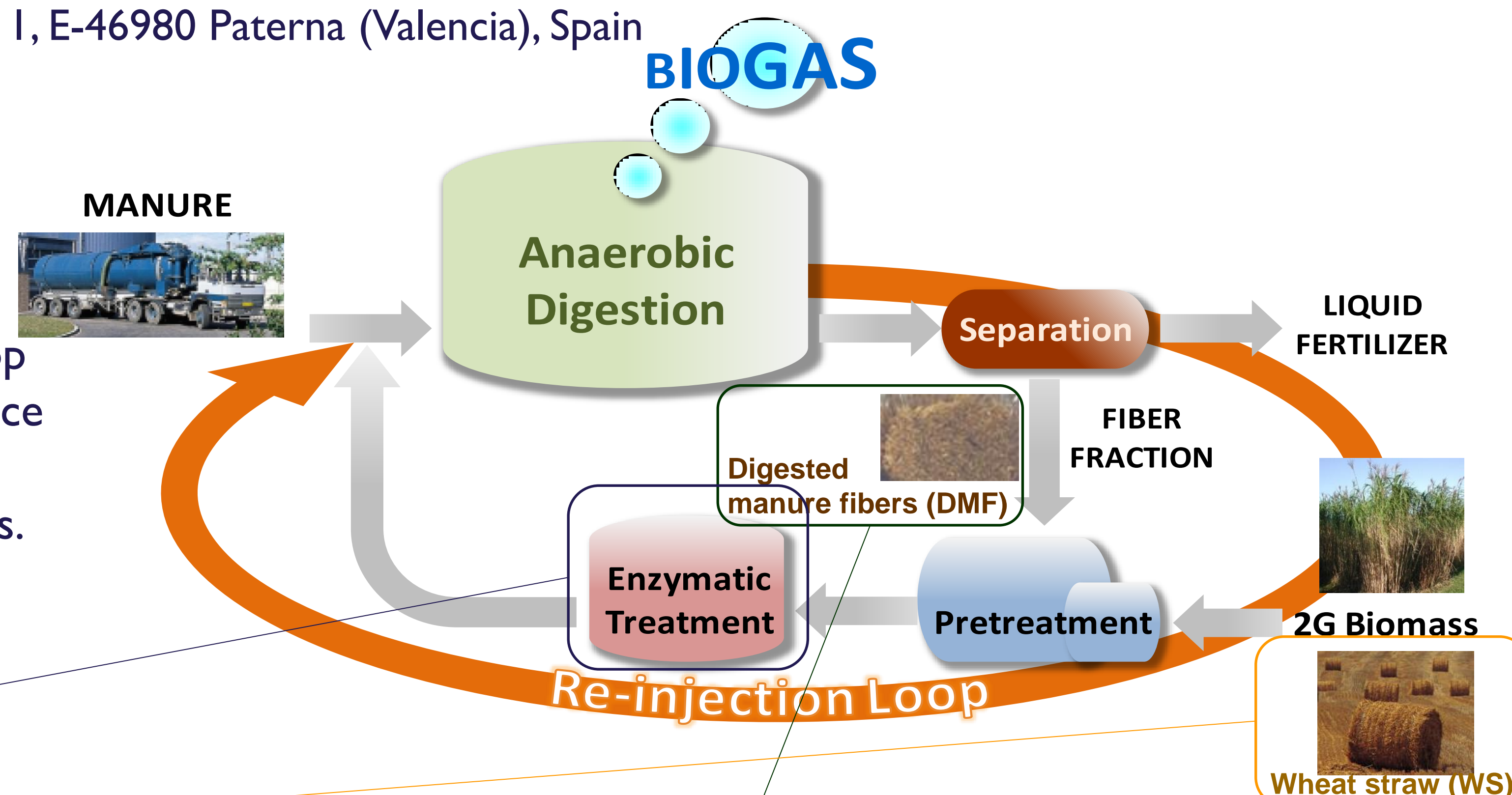
<sup>3</sup> ainia centro tecnológico, Parque Tecnológico de Valencia c. Benjamin Franklin 5-1 I, E-46980 Paterna (Valencia), Spain

\*Corresponding author, ph +45 9940 2585, E-mail address: [hu@bio.aau.dk](mailto:hu@bio.aau.dk)

## The Re-Injection Loop Concept

In this study, enzymatic treatment was tested to increase the biogas yield of wheat straw (WS) and digested manure fibers (DMF) in the Re-Injection Loop Concept, which combines anaerobic digestion with solid separation to enhance the biogas yield per ton of manure by:

1. Digestion of the easily degradable fraction of manure in the biogas process.
2. Separation of the residual recalcitrant digested fiber fraction project.
3. Ultrasound and/or enzymatic treatment of the digested fiber fraction.
4. Recirculation of the treated fiber fraction into the reactor.



## Enzymatic Treatment of WS and DMF

- All tests were conducted in batch tests as separate enzymatic hydrolysis prior to the anaerobic digestion (AD) process. The enzymatic hydrolysis (EH) was performed at the following conditions: T = 50°C,  $\tau$  = 0.5/1.0/72 h, dosage = 0.1/2.5/5.0% g-enzyme/g-TS, 10% TS, pH (adjusted) = pH 5/7, pH (non adjusted) = pH > 8

- The following enzyme blends were tested:

Enzyme	Activity	pH range	Temp range (°C)
NZ-P	Protease	8.0-10.0	60-70
ES-C5000P	Neutral cellulase	6.5-8.5	50-70
ES-CX15K	Cellulase and endo-xylanase	2.5-7.0	40-70
NZ-Cat	Catalase	N/D	N/D
NZ-CBG	Cellulase	4.5-6.0	40-55
ES-HC	Endo-xylanase	5.0-8.0	50-80
NZ-CBG1	Cellulase	4.0-6.0	50
ES-C-LA	$\beta$ -glucanase & endo-cellulase	4.5-6.0	40-50
NZ-X	Xylanase	4.5-6.1	50-70
NZ-BG	$\beta$ -glucanase	5.0-7.0	50-65
ES-CX900T	Cellulase + xylanase	6.5-8.0	50-70
ES-3000L	Cellulase	4.0-7.0	30-60
ES-8000P	Cellulase	4.5-6.5	50-60

NZ: enzyme blend from Novozymes A/S, ES: enzyme blend from EnzymeSupplies Ltd.

- The subsequent biomethane potential (BMP) tests were performed at 37°C for 60 days. The inoculum used was taken from Hashøj biogas plant, Denmark, treating manure and industrial organic waste.

- Screening of the different enzymes (50°C, pH 5.0, 72h) on WS showed an increasing effect on the BMP mainly for enzyme blends containing both cellulase and xylanase activity:

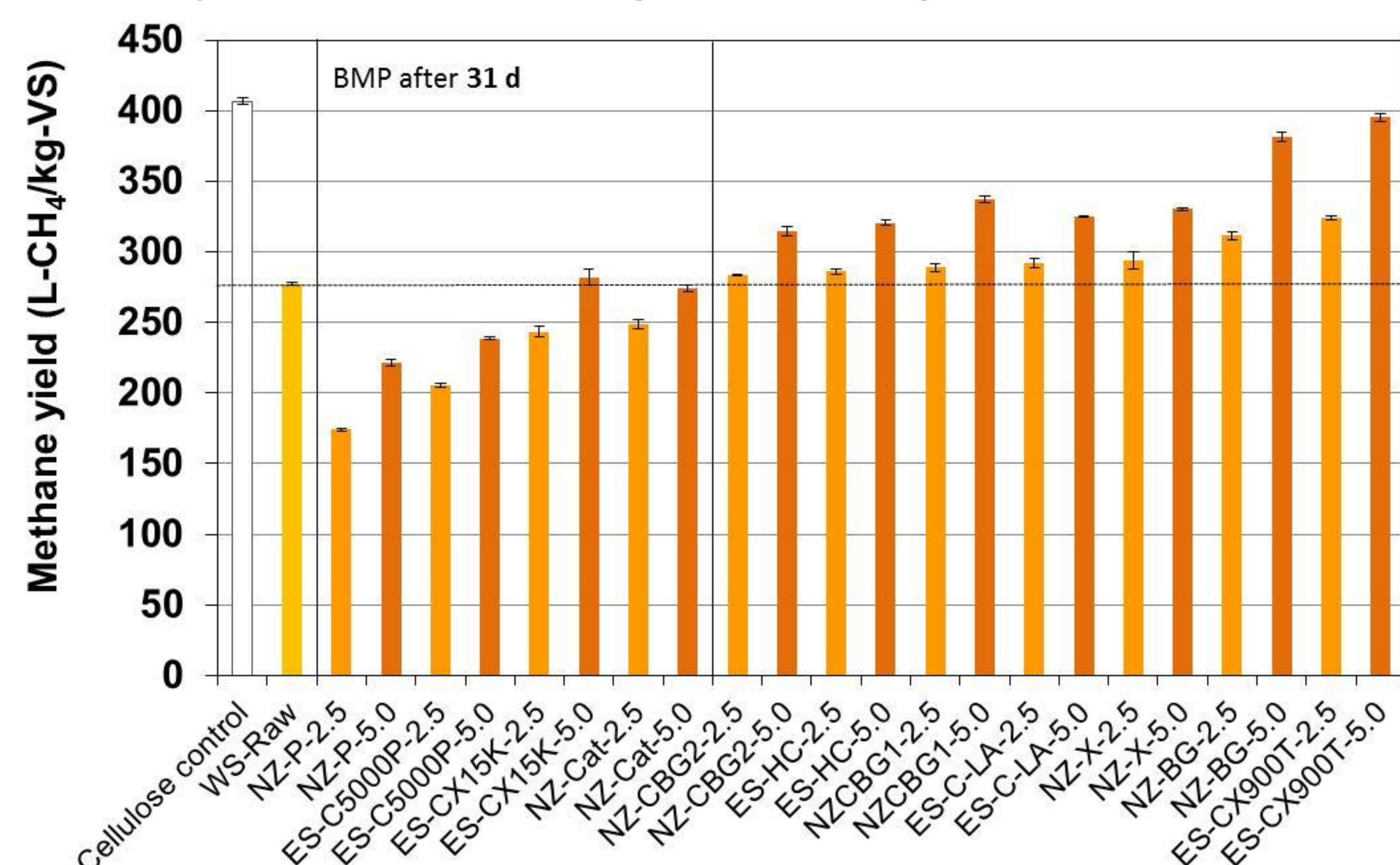


Fig.1 BMP of WS using different enzyme blends in 2.5/5.0% g-enz/g-TS compared to raw WS (—)

- EH (50°C, pH 7.0/5.0, 0.5/72h) using the best performing and low cost enzyme blends (CX900T/3000L) at low dosage (0.1% g-enzyme/g-TS) had an increasing effect mainly on the BMP of DMF, while the effect on WS was only limited:

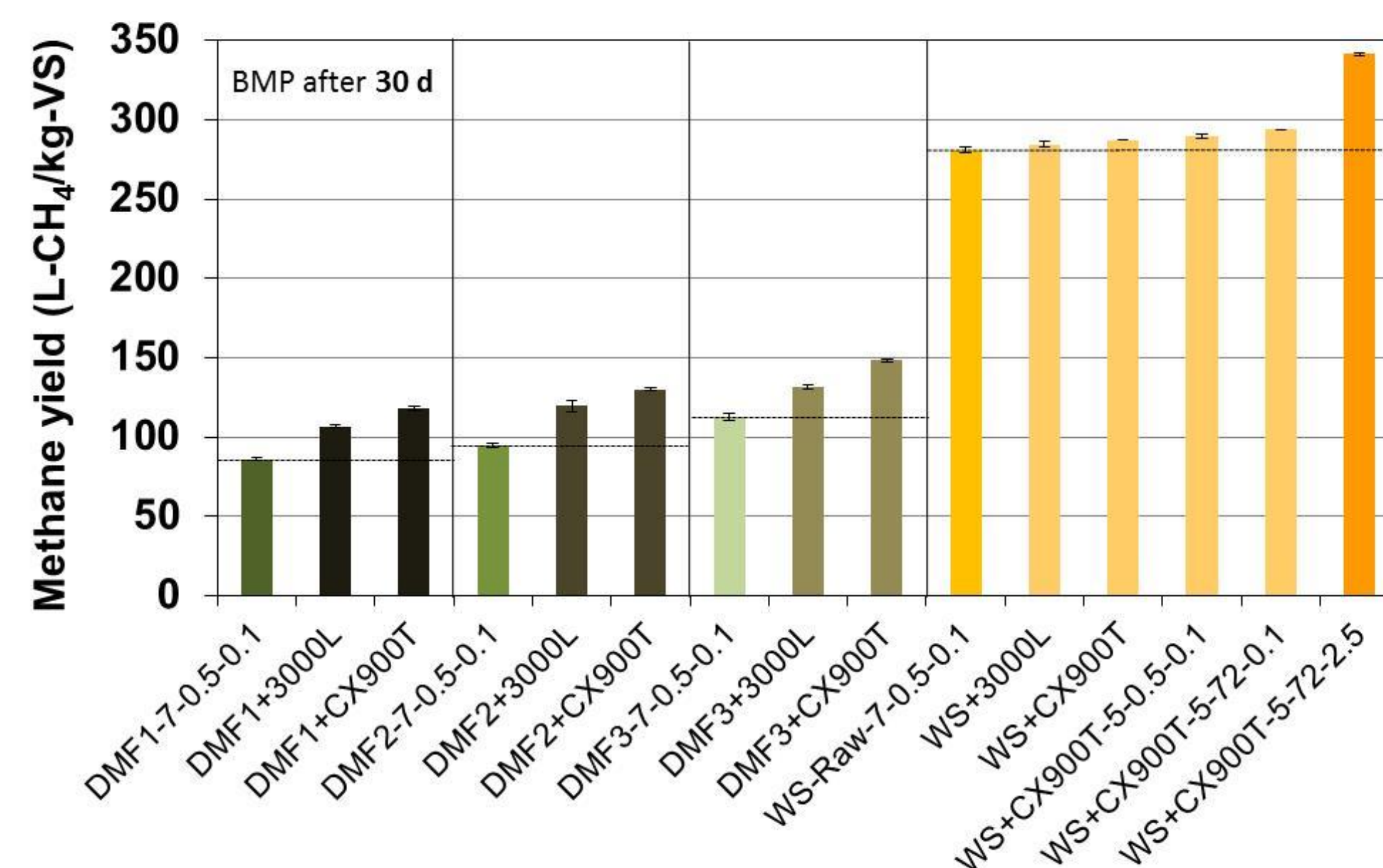


Fig. 2 Effect of enzyme addition on BMP of DMF from 3 different biogas plants (DMF1-3) and of WS

- EH with no pH adjustment (pH > 8) and 0.1% dosage showed an increasing effect of the enzyme addition (8000P) compared to treating DMF at 42/50°C without enzymes only for 72h of EH:

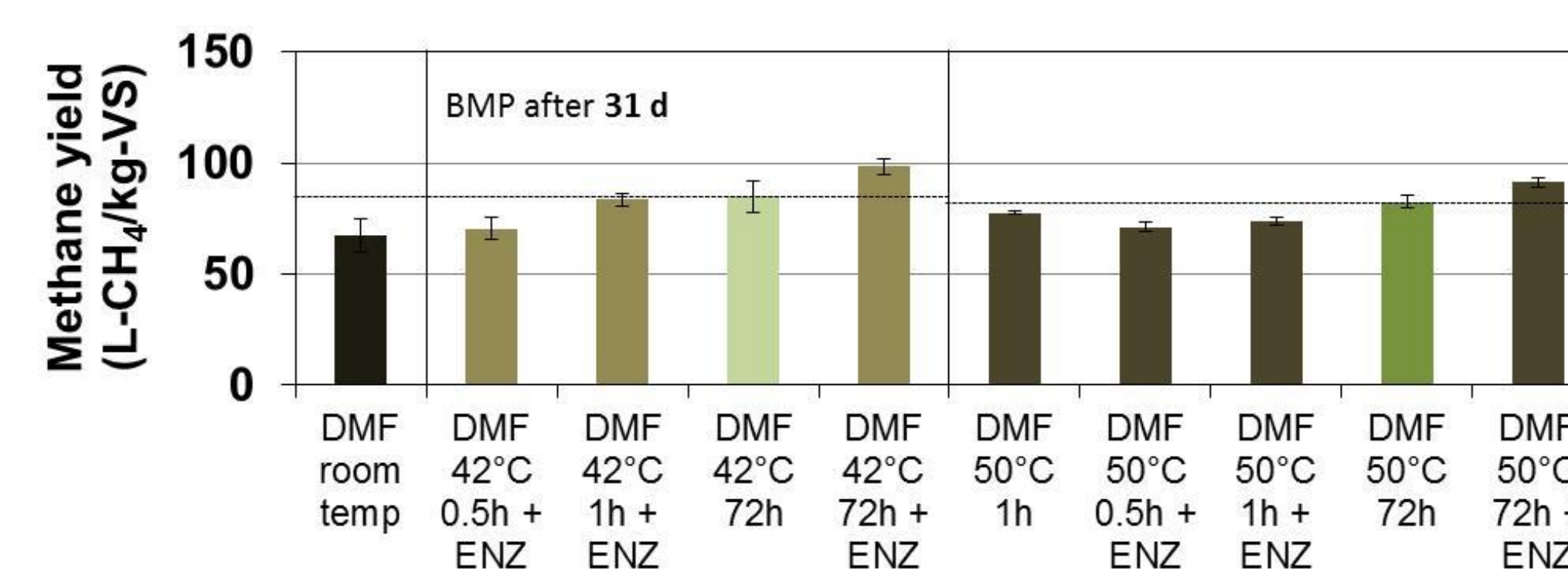


Fig. 3 BMP of DMF after treatment at 42/50°C with and without enzyme addition (for 72h —)

## Conclusions

- Addition of combined cellulase and xylanase activity showed highest effect to enhance the intrinsic hydrolytic activity of the AD process
- The relative effect of enzyme addition was higher for DMF with low BMP
- pH adjustment to pH < 7 showed higher effect of the enzyme addition

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