

# Application of multi-enzymatic hydrolysis for improving the efficiency of the biogas production in solid waste fermentation process in Ostróda WWTP

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**Abstract.** Biomass fermentation is one of the important sources of renewable energy in EU. Application of multi-enzymatic hydrolysis process enables a significant increase in efficiency of biogas production. The main goal of the paper is to present the results of the pilot scale research performed in WWTP in Ostróda. The fixed combination of three enzymes was continuously introduced: amylase, lipase and protease. Research aimed at verifying the impact of enzyme dose on sludge digestion process and on the amount of biogas produced. Statistical analysis of the research results allows to determine the influence of dosing the enzymes in mesophilic digestion on the biogas production.

## 1 Introduction

Sewage sludge is an inherent product of the operation on WWTP. The most common method of stabilization is anaerobic digestion. The efficient production and use of biogas in a WWTP is a source of heat and power. Multi-enzymatic hydrolysis is a biological technology of primary disintegration of sewage sludge. It accelerates and improves the efficiency of the natural process of hydrolysis [1] occurring in the first stage of sludge digestion, uses a number of naturally produced [2] or additionally dosed forms of [3] enzymes: amylase, protease [4] and lipase [5], which reduce the activation energy of the hydrolysis of selected organic compounds. As a result, the percentage of biodegradable organic matter increases, which provokes the increase in the efficiency of biogas production.

The aim of the study was to determine whether the method of multi-enzymatic hydrolysis improves the efficiency of the sludge digestion process from the treatment plant in Ostróda and if it increases the biogas production during the mesophilic digestion.

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## 2 Materials and methods

The research was conducted in a pilot scale in the period 05.10.2011–31.07.2012 in the Ostróda WWTP belonging to PWiK Ostróda sp. z. o.o.

There were three series of tests:

- Series I. Start-up and stabilization of the mesophilic digestion process in the period of 05.10.2011–08.12.2011
- Series II. Sludge mesophilic digestion with the dosage of the enzymes with 20% of dry solids concentration, in the period of 09.12.2011–26.03.2012
- Series III. Sludge mesophilic digestion with the dosage of the enzymes with 10% of dry solids concentration in the period of 27.03.2012–31.07.2012

The model consisted of a feed tank with a capacity of 1130 litres for the continuously supplied primary and excess sludge, mixed in equal proportions. The sludge from the feed tank was directed to a digester with a capacity of 1410 litres. In Series I the temperature in the digester was 38°C, HRT = 35 days. In the Series II and III, the enzymes were dosed to the feed tank in an amount of 20 and 10% dry mass. In the Series III, the temperature was lowered to 35°C and the sludge HRT was reduced to 29 days. Digested sludge was directed to the final tank.

The scope of the research included the measurement of the parameters in raw and digested sludge and the volume of produced biogas. The daily volume of biogas was measured and calculated to the volume of the biogas produced from dry organic mass contents ( $Q_{ODM_{INF}}$ ). For each day of the test, the digestion index was calculated. This index demonstrates the effectiveness of decomposition of organic compounds in the sludge.

Samples were collected at the feed tank (mixed sludge) and the final tank (digested sludge).

The analysis of sludge parameters were made according to the following standards:

- Determination of dry matter PN-EN 12880:2004
- Determination of the loss of ignition of dry mass % – PN-EN 12879:2004
- Determination of COD - PN-74/C-04578.03

## 3 Results

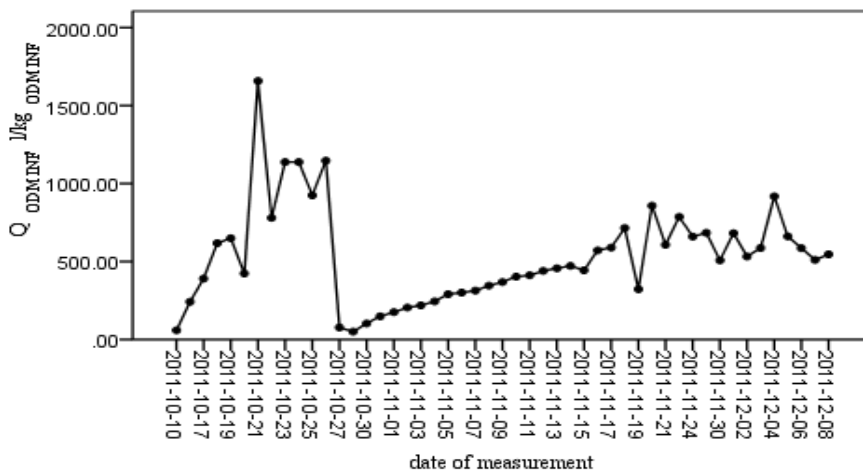
### 3.1 Series I – start-up and stabilization of mesophilic digestion process

After the start-up, the reactor worked in batch process and then in continuous one. The temperature in reactor was 38°C, and the HRT was 35 days. Table 1 shows the sludge quality before and after digestion process during series I and the calculated digestion index.

At the beginning of the research, the dry matter content (DM) in the raw sludge was in the range of 5–6%. The content of the organic dry matter (ODM) remained at a high level oscillating around 78-80%. The COD value was usually over 60 000 mg/L. The dry matter content of digested sludge, which first was low (1.7%), experienced a period of rapid growth to a 4.3%, then declined and finally began to stabilise at around 3.1%. The content of dry organic matter decreased from approximately 73% (the beginning of the study) to about 63%. The actual value of the digestion index, at the start of work is very low 2–6.5% and next grew and ranged between 42.8–56.7. The COD value decreased from 56 000 mg/L (the beginning of the study) and was about 32 000 mg/L.

**Table 1.** shows the sludge quality before and after digestion process during series I and the calculated digestion index.

Date	Sludge						
	From feeding tank			After digestion			
	DM	ODM	COD	DM	ODM	COD	Digestion index
	%	%DM	mg/L	%	%DM	mg/L	%
2011-10-17	6.1	77.0	60 000	1.7	75.8	-	6.5
2011-10-25	5.6	73.7	32 100	4.3	73.3	56000	2.0
2011-11-15	5.6	76.3	60 000	3.3	63.6	31400	45.7
2011-11-22	5.0	78.7	60 000	3.1	63.9	32800	42.8
2011-11-24	5.1	78.2	59 600	3.1	64.0	31900	44.6
2011-11-29	4.9	79.7	60 000	3.1	63.7	31400	49.7
2011-12-01	5.2	80.0	60 000	3.1	63.4	32600	53.6
2011-12-06	5.2	80.7	60 000	3.1	62.4	32400	56.7



**Fig. 1.** The volume of biogas produced in the Series I.

In the series I, a far reaching variation of biogas production might be observed (Fig. 1). The stabilisation of the digestion process was preceded by some significant changes in the biogas production expressed by  $Q_{ODM\ INF}$  400–900  $l/kg_{ODM\ INF}$ .

### 3.2 Series II – enzyme dosage of 20% contents dry solid

Before the start of the series II, a stabilization work without the addition of enzymes was achieved. Thus, decisions have been taken about dosage of enzymes. Enzymes were dosed directly into the feed tank in an amount of 20% of dry matter. Table 2 shows the sludge quality before and after the digestion process during series II and the calculated digestion index.

The dry matter content in the raw sludge in series II showed considerable variation, the trend of decreasing the level of 5–6% to 3–4%. The content of organic dry matter remained at a high level oscillating between 78% and 85%, but in this case, at the end of the

experiment, the occurrence of a significant downward trend could be observed. Sludge COD value was located outside the range of the measurement (60000 mg/L) not as often as in the series I. In February, in a period of intense cold, the sludge COD dropped significantly to the level of 30800 mg/L. Later, it returned to the level of 40000–50000 mg/L.

The decrease in dry matter content in the raw sludge caused the decline in dry matter content in sludge after the mesophilic digestion process to the level of 2.4–3.2%. The content of dry organic matter in the digested sludge was 62–63% before starting the dosage of enzymes and increased to 64–68% as a result of dosing enzymes. The calculated actual digestion index has a high variability within the range of 27.3–65.3% (average 52.1%).

COD value of digested sludge remained relatively stable compared to the changing raw sludge that was supplied. However, it decreased from above 28600 mg /L from the period before the frost to the level of 24100–27400 mg/L.

**Table 2.** Parameters in the raw and digested sludge obtained in the series II.

Date	Sludge						
	From feeding tank			After digestion			
	DM	ODM	COD	DM	ODM	COD	Digestion index
	%	% DM	mg/L	%	%DM	mg/L	%
2011-12-13	5.0	82.8	60 000	3.0	63.9	32300	58.9
2011-12-20	5.2	78.5	60 000	3.2	62.4	31300	61.7
2011-12-27	4.9	82.4	60 000	3.0	62.8	31400	64.5
2012-01-03	4.7	83.4	58 500	3.1	63.2	31600	65.8
Sludge digestion with the dosage of the enzymes with 20% of dry solids concentration							
2012-01-10	4.6	82.1	54 400	3.0	64.7	37000	37.7
2012-01-17	4.9	82.0	58 400	2.9	64.6	26000	37.7
2012-01-24	5.4	83.9	37 800	2.9	66.4	10600	38.5
31.01.2012-13.02.2012 The period of very low temperatures							
2012-01-31	5.6	84.9	60 000	2.9	66.5	28900	41.3
2012-02-07	4.0	84.3	48 300	2.9	67.1	27800	37.8
2012-02-14	5.1	84.9	60 000	2.8	68.5	26700	35.7
2012-02-21	6.0	84.4	60 000	2.8	68.0	27000	35.5
2012-02-28	4.5	82.2	51 100	2.6	67.8	29300	28.8
2012-03-06	4.0	82.4	52 100	2.8	65.1	26400	44.6
2012-03-08	3.0	76.5	30 800	2.4	67.6	24100	8.7
2012-03-13	2.9	79.9	41 200	2.7	63.6	25700	49.9
2012-03-22	3.7	77.9	46 600	2.6	66.1	27400	51.5

In the Series II there was an increase in the average daily production of biogas during the dosing of enzymes (Fig. 2). The significance of this observation becomes apparent when comparing the value of  $Q_{ODM INF}$  before and after enzyme dosing. The efficiency of biogas production increased from an average of 600 l/kg of dry organic matter to the level of 900 l/kg of dry organic matter. In addition, it was observed that although initially the decline in the content of the raw sludge dry mass was not included in the enzymes dosage which therefore exceeded 20%, significant differences in the biogas production efficiency were not observed. The dose of enzymes, which was calculated based on previous laboratory tests, could therefore be too high. That is why the dose of enzymes with 20% to 10% dry solid concentration was changed, in order to optimise the dose in the future.

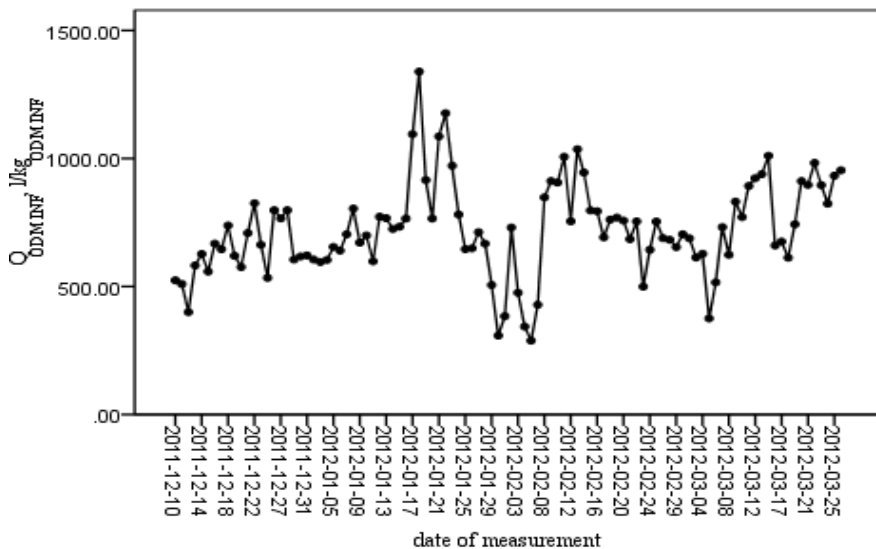


Fig. 2. The volume of biogas produced in the Series II.

### 3.3 Series III – enzyme dosage of 10% contents of dry solid

The purpose of Series III is to check if a lower dose of enzymes (content 10% dry solid) will have a comparable effect on the efficiency of biogas production. In this series, the sludge HRT was reduced to 29 days, and the temperature was lowered to 35°C. Table 3 shows the sludge quality before and after the digestion process during Series III and the calculated unit of fermentation.

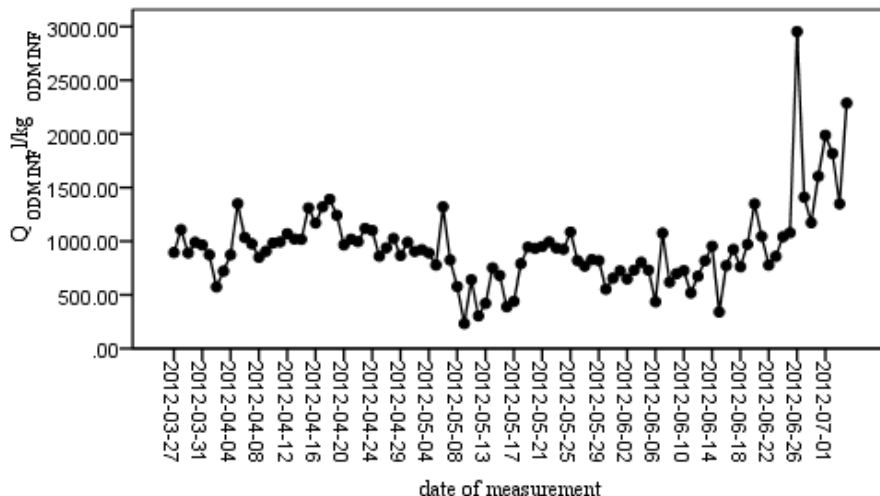
Dry matter content in the raw sludge in the Series III showed a large variation in the range of 2.1–5.9%. In this period, the organic dry matter content in the raw sludge was 80%. A noticeable decrease to 65.3% was recorded in the summer. In July, the dry matter content significantly increased – more than 5% while the content of organic dry matter decreased to less than 70%. Sludge COD value dropped to the level of 27700 mg/L, then rose to the level of the maximum measurable excess of 60000 mg/L.

Digestion conditions during the Series III remained stable, despite the changes in the quality of raw sludge. The dry matter content was in the range of 2.2–3.6%. The dry organic matter was lower than in the Series II – in the range of 54.7–63.3%. The calculated actual digestion index exceeds 50%. The value of COD of the sludge was in the range 19000–30500 mg/L.

**Table 3.** Parameters in the raw and digested sludge obtained in the Series III.

Date	Sludge						
	From feeding tank			After digestion			
	DM	ODM	COD	DM	ODM	COD	Digestion index
	%	% DM	mg/l	%	% DM	mg/l	%
2012-03-29	4.4	78.8	54200	2.6	62.0	30 500	58.4
2012-04-10	3.3	79.2	41400	2.5	63.3	25 000	52.0
2012-04-17	2.1	72.4	27700	2.4	61.7	25 300	53.3
Sludge digestion with the dosage of the enzymes with 10% of dry solids concentration							
2012-04-24	4.2	80.8	60000	2.4	59.6	29 100	53.4
2012-05-08	2.8	78.9	42300	2.3	62.0	21 900	50.3
2012-05-15	2.7	76.4	40000	2.5	59.2	19 000	55.5
2012-05-22	2.7	77.3	36200	2.5	57.9	21 700	45.7
2012-05-29	3.0	78.3	50100	2.3	59.9	22 600	58.6
2012-06-05	3.8	77.0	53800	2.4	58.4	29 000	58.1
Batch work							
2012-06-12	3.0	77.8	38300	2.2	62.2	23 400	53.0
2012-06-22	4.2	73.8	61200	2.6	54.7	22 800	57.1
2012-06-28	5.9	73.6	60000	2.8	53.9	24 100	58.1
2012-07-05	3.9	69.6	41400	3.0	56.6	27 000	43.0
2012-07-13	5.0	70.9	60000	3.1	55.8	26 000	48.2
2012-07-19	5.4	66.5	60000	3.2	54.7	28 600	39.2
2012-07-26	5.2	65.3	60000	3.6	62.2	28 100	12.6

In the Series III there was a decrease in the average daily production of biogas because the dry matter content in the raw sludge decreased. Organic compounds are the material for methanogenic bacteria. The decrease in the enzyme dose could cause a  $Q_{ODM INF}$  decrease, which could be associated with the lower efficiency of multi- enzymatic hydrolysis process. Finally, reducing HRT from 35 to 29 days could reduce the efficiency of biogas production. The reasons for the decline in production are therefore complex and require further analysis.



**Fig. 3.** The volume of biogas produced in the Series III.

During the series III we have managed to achieve an intriguing but inconclusive research. Double dose reduction enzymes with 20% contents of dry matter to 10%, influenced the course of the process, but in a decidedly ambiguous way. The amount of biogas produced  $Q_{ODM\_INF}$  in Series II and III was on the same level.

## 4 Discussion

### *Verification of hypotheses*

To systematize conducted analysis, focused on the influence of multi-enzymatic hydrolysis on the efficiency of biogas production, two following research hypotheses were elaborated.

**H I.** In the process of mesophilic sludge digestion, enzyme dosing increases the efficiency of biogas production.

In the first stage of hypotheses verification, mean values and standard deviation of  $Q_{ODM\_INF}$  were compared. In the series II and III (dosing of enzymes) higher mean value  $Q_{ODM\_INF}$ . The difference of  $Q_{ODM\_INF}$  value in series was initially estimated at 219.7 l/kg  $Q_{ODM\_INF}$  (34.5% increase).

**Table 4.** Biogas production  $Q_{ODM\_INF}$  and enzymes dosing.

Dosage of the enzymes	Mean $Q_{ODM\_INF}$	Standard Deviation
No (Series I)	636.0 l/kg $Q_{ODM\_INF}$	122.6 l/kg $Q_{ODM\_INF}$
Yes (Series II and III)	855.7 l/kg $Q_{ODM\_INF}$	331.2 l/kg $Q_{ODM\_INF}$

Second stage of the analysis in the process of verification of potential relation between variables was generating a model of linear regression and testing the statistical significance of generated model. Statistically important model ( $\chi^2 = 0.000$ ,  $R^2 = 0.086$ , ANOVA significance = 0.000) was obtained. It allowed to estimate following linear regression equation, assessing statistically important, positive influence of enzyme dosing on biogas production in mesophilic digestion.

$$Q_{ODM\ INF} = 636.0 + 219.7 \cdot e [l/kg_{ODM\ INF}] \quad (1)$$

e: dosing of enzymes (0 = no; 1 = yes) (dummy variable)

**H II.** In the process of mesophilic sludge digestion, linear effect of enzyme dosing on biogas production is observed.

To assess the size of optimal enzymes dose, mean values and standard deviations of  $Q_{ODM\ INF}$  for different sizes of doses effects were compared. For analysed data, the highest level of  $Q_{ODM\ INF}$  can be observed for size of dose 10% of dry solids concentration in fermentation chamber.

**Table 5.** Biogas production  $Q_{ODM\ INF}$  and size of enzymes dose.

Size of enzyme dosage	Mean $Q_{ODM\ INF}$	Standard Deviation
no dosage	636.0 l/kg $_{ODM\ INF}$	122.6 l/kg $_{ODM\ INF}$
10% of dry solids concentration	899.1 l/kg $_{ODM\ INF}$	411.7 l/kg $_{ODM\ INF}$
20% of dry solids concentration	827.1 l/kg $_{ODM\ INF}$	263.2 l/kg $_{ODM\ INF}$

To verify observation, model of linear regression was generated. Preliminary analysis suggested considerably low explanatory potential of the linear model of enzyme dose influence (  $\text{Chi}^2 = 0.001$ ;  $R^2 = 0.037$ ; ANOVA significance = 0.001). To analyse the potentially non-linear relation between size of enzymes dose and biogas production, the size of dose data were recoded into two 0–1 “dummy variables” for each size of enzyme dosage:  $e_{20}$  (20% of dry solids concentration) and  $e_{10}$  (10% of dry solids concentration). The second model that was generated was statistically significant ( $\text{Chi}^2 = 0.000$ ,  $R^2 = 0.096$ , ANOVA significance = 0.000). It allowed to estimate following linear regression equation, assessing statically important, positive influence of size of dose of enzymes on biogas production in mesophylic digestion of sewage sludge.

$$Q_{ODM\ INF} = 636,0 + 263,1 \cdot e_{10} + 191,1 \cdot e_{20} [l/kg_{ODM\ INF}] \quad (2)$$

$e_{10}$ : Enzyme dose 10% of dry solids concentration (0=no) (1=yes) (dummy variable)

$e_{20}$ : Enzyme dose 20% of dry solids concentration (0=no) (1=yes) (dummy variable)

The production of biogas was proven more effective for 10% dry solids enzyme dose. The relation between variables was presented with three equations, representing separate influence of 10% ds and 20% DM enzyme dose. The positive influence of optimal dose of enzymes, 10% of dry solids, on biogas production was estimated on 263.1 l/kg $_{ODM\ INF}$  (41% increase). Last equation,  $Q_{ODM\ INF\_e10}$ , represents the negative influence of enzymes over the optimal 10% dry solids dose.

$$Q_{ODM\ INF} = 636.0 + 263.1 \cdot e_{10} [l/kg_{ODM\ INF}] \quad (3)$$

$$Q_{ODM\ INF} = 636.0 + 191.1 \cdot e_{20} [l/kg_{ODM\ INF}] \quad (4)$$

$$Q_{ODM\ INF\_E10} = 899.1 - 191.1 \cdot e_{20} [l/kg_{ODM\ INF}] \quad (5)$$



## 5 Conclusion

Studies show that there is a positive relationship between enzyme dosing and the increased productivity of biogas production in mesophilic fermentation. The results allow us to estimate that the enzyme dosage allows for an increase in the production of biogas from kilogram of organic dry mass of 48%. The analyses indicated that in the case of mesophile fermentation, statistically there is no significant relationship between the increase of enzyme dose and the increase of biogas production. The data obtained suggests that the increase in the dose of more than 10% of dry mass may even cause a decrease in biogas production.

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