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Low temperature BMP tests using fish waste from invasive Round goby of the Baltic Sea

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Abstract. Round goby *(Neogobius melanostomus*) is an invasive fish species in the Baltic Sea. While meat can be used for human consumption, fish processing residues are considered as a waste. Within circular economy and bio-economy perspectives fish waste could be used as a valuable feedstock for biogas production. However, the research is mostly focused on evaluating biogas yield at mesophilic conditions (i.e. 37 °C). In this study the impact of low temperature on Biochemical Methane Potential (BMP) tests has been investigated. Round goby's processing leftovers - heads, intestines and skin/bone mixture were tested in codigestion with sewage sludge. Anaerobic digestion (AD) was carried out in 100 mL batch tests at low temperature 23 °C and 37 °C conditions, over an incubation period of 31 days. The results show that AD at low temperature occurs twice as slowly as under 37 °C conditions. However, after 31 days the BMP values for 23 °C samples were only 2% lower than for high temperature samples. Heads and skins showed similar BMP values reaching on average 502 L CH_4 kg_{VS}^{-1} and 556 L CH_4 kg_{VS}^{-1} respectively. BMP for fish intestines was higher, reaching on average 870 L CH_4 kg_{VS}^{-1}. Average BMP for mixes of fish heads, skins, intestines and bones was 660 L CH_4 kg_{VS}^{-1}. Acquired BMPs were further compared with the theoretical BMPs from Buswell's formula. Research results suggests that anaerobic digestion of fish waste under low temperature conditions could be feasible as the process still efficiently occurs, in fact opening a new opportunity to explore the overall sustainability of technologies based on these conversion processes.

Key words: Biomethane, low temperature, fish waste, anaerobic digestion, Neogobius melanostomus.

INTRODUCTION

In last decades' the population of round goby (*Neogobius melanostomus*) has spread into the Baltic Sea. Coming from Caspian Sea, this fish in Latvian coastline has been firstly observed in 2004 and since then, the amount of it has increased significantly reaching 25 tons in the year 2013 and more than 700 tons in year 2017 (Riekstiņš, 2014; 2017). Currently in the nearshore waters of the Baltic Sea this is the second most caught fish species after the Baltic herring. Distribution area is still expanding and has become a huge problem regarding both environmental and economic aspects. This fish species has become invasive in Latvia due to easy adaption to surrounding environment (Charlebois et al., 2001). Since the amount of fish has been growing, it can represent a valuable economic opportunity.

Physiology of round goby allows using only 40% of it as a meat for food, creating large amounts of waste. Waste biomass includes parts like skin, head, bones, fins and intestines (Eiroa et al., 2012). In recent years potential use of this fish waste has become a popular research topic. Melvere et al. (2017) describe many options for use of round goby's processing waste in bioeconomy. The author suggests using it as raw material to produce a wide range of products including also high value-added end products like enzymes, proteins and fish oil. Salam et al. (2009) claims that fish waste can also be successfully used for energy production producing biogas in anaerobic fermentation processes. However the high content of ammonia nitrogen might negatively affect fermentation processes thus one of the best ways for fish waste biomethanation is co-digestion (Tomczak-Wandzel et al., 2013).

Anaerobic fermentation has been used for waste treatment and biogas recovery from many types of organic waste. Its numerous advantages, such as the recovery of a renewable energy, waste volume and odour reduction are well documented (Gunaseelan, 1997; Wu et al., 2009). Anaerobic treatment of fish waste not only reduces unpleasant odour but also gives the opportunity to regain some energy used for the production processes. However, until recently, research has mainly been focused on anaerobic digestion (AD) at mesophilic (25–45 °C) or thermophilic (45–65 °C) temperatures. It is believed that a lower temperature in the psychrophilic range (< 25 °C) reduces microbial activity and in fact is lowering the biogas yield (Connaughton et al., 2006; Saady & Massé, 2013). One of the main advantages of psychrophilic temperatures would be the lower energy input required for heating the reactor, consequently reducing the overall operating cost (Smith et al., 2013). The most recent results on microbiological activity in psychrophilic conditions show that lower temperatures require a longer fermentation time and lead to higher methane content and lower accumulation of volatile fatty acids compared to mesophilic conditions, although still keeping a similar cumulative biomethane yield in both conditions (Wei & Guo, 2018).

In this study experiments on biogas production at mesophilic and lower temperatures were carried out and the data have been compared. The aim of this study was to assess the process performance of two BMP test setups inoculated with the same sewage sludge for treatment of fish waste. One setup of 100 mL bioreactors was operated at 37 °C, while the second was maintained at room temperature 21–23 °C. Comparative investigations of biomethane production in both temperature ranges would allow evaluation of the overall economic feasibility. In fact, it would be a key aspect to assess the potential benefits in operational costs in terms of lower energy input required for heating, reduction of the amount of waste in fish processing plants, energy recovery capability within the production processes, although bigger digester volume may be necessary.

MATERIALS AND METHODS

Substrate (collection, pre-treatment, and storage)

The *Neogobius melanostomus* used within the batch tests for the BMP evaluation were freshly caught on Baltic sea costal area in August 2015 (biomass 2) and April 2017 (biomass 1), near the city of Liepaja, West Latvia. Whole fish samples were transported within plastic bags to the Biosystem Laboratory at the Riga Technical University, separated in smaller portions and then frozen at –18 °C. Prior experiments biomass was defrosted at room temperature. Then fish were skinned, gutted, deboned and beheaded. Processing waste products – heads, intestines and skin/bone mixture were used for further BMP testing. Each fish waste fraction was separately homogenized using 1,500 W kitchen blender and given to total solids (TS) and volatile solids (VS) content analyses. Homogenized samples were frozen again at –18 °C, and defrosted a day before the start of BMP tests.

TS and VS values were determined prior to the experiments based on ISO Standards (ISO 14780:2017, ISO 18134–2:2017, ISO 18134–3:2015). TS were obtained by placing a sample into an oven for 18 hours at 105 °C, and then the dry sample was finely ground and placed into an oven for 5 hours at 105 °C. VS were obtained by placing 5g of totally dry sample into an oven for 11 hours with a heating step 50 °C h^{-1} and then kept at 550 °C for 3 hours to be able to obtain the VS content as a fraction of TS (% of TS). The results are presented in Table 1.

Table 1. TS and VS content of inoculum and fish waste fractions

Substrate	TS, %	VS, % of TS
Inoculum 1	2.0	60.5
Inoculum 2	1.9	60.5
Inoculum 3	1.9	60.5
Heads[1]	20.5	76.5
Skin/bone mix[1]	22.2	75.3
Intestines[1]	36.7	82.6
Heads[2]	19.8	76.5
Skin/bone mix[2]	19.4	75.3
Intestines[2]	30.1	82.6

Inoculums 1, 2, 3 – inoculums for experiment 1, 2 and 3; [1] – biomass 1; [2] – biomass 2.

Inoculum

Sewage sludge was collected from local waste water treatment plant 'Daugavgriva' (Riga district, Latvia) directly from biogas bioreactors. Prior to the BMP experiments, the inoculum was incubated for 6 days at 37 °C, with regular degassing. Inoculum was always evaluated for TS and VS content using ISO standards (ISO 14780:2017, ISO 18134–2:2017, ISO 18134–3:2015, ISO 18122:2015).

BMP test method

BMP tests were used to define the amount of methane produced per kilogram of VS, for an inoculum to substrate ratio (ISR) equal to 3 based on a TS basis. Generally, BMP measuring methods are based on liquid displacement or the displacement of a syringe piston. An alkaline solution for cleaning the biogas (by absorbing the CO_2 fraction) is added in both methods. The method is a well-known approach, but still lacking true standardization (Esposito et al., 2012; Edward et al., 2015). A pH range from 6.5 to 8.2 (Ağdağ & Sponza, 2005; Chandra et al., 2012; Esposito et al., 2012) is optimal for most anaerobic bacteria, including methanogens. Therefore, an alkaline compound is normally added within the solution as a buffer capacity (i.e. sodium hydroxide, sodium (bi)carbonate or sodium sulphide) (Chynoweth et al., 2000), in our case a 0.7M $NaHCO_3$ solution was specifically prepared.

BMP is a sensitive method, influenced by the conditions for the anaerobic bacteria to grow. In this light, the analysis of the results can be difficult due to the amount of potentially influential factors, resulting in likely possible errors and/or inaccuracies (Angelidaki & Sanders, 2004, Wellinger et al., 2013). Moreover, sometimes the same substrates don't show the same BMPs based on the tests' conditions (Del Borghi et al., 1999).

Experimental set-up

BMP tests were conducted in a batch mode using 100 mL crimp neck ND20 vials with a working volume of 50 mL. Each bottle was filled with 30 mL of distilled water, 20 mL of inoculum and 1mL of 0.7M $NaHCO_3$ buffer basal solution to maintain neutral the pH. Different amount (fresh weight) of different fish waste fraction was added to specific samples based on TS content and to maintain ISR around 3. Additionally, reference samples (blanks) containing only inoculum were prepared both for high and low temperature conditions to account for the methane production solely from the fish waste biodegradation. Sample headspace was flushed with N_2 for 30 seconds at flow rate around 2 L min^{-1} before sealing them with butyl rubber stoppers and aluminium crimps. The tests were carried out in dark conditions at a mesophilic temperature (37 °C) in the EcoCell LSIS-B2V / EC 111 incubator and at 23 °C, and lasted for 31 days. The batches were manually shaken one time per day on average. All batch tests were prepared in triplicates.

In total, three experiments were performed. In first experiment fish waste from year 2017 (biomass 1) was used. Tested samples contained heads, skin/bone mixture and intestines. For second and third experiment fish waste from year 2015 (biomass 2) was used. These samples also contained heads, skin/bone mixture, intestines and additional biomass mixes (consisting of all waste fractions in different shares). First mix (M1) contained all waste fractions in equal share based on TS. Second mix (M2) contained all waste fractions in equal share based on wet weight. Third mix (M3) contained all waste fractions in wet weight ratios: 2 parts heads, 2 parts skin/bone mixture, 1part intestines (based on practical fish processing approach when intestines make up only one fifth of total waste amount). Experiments were performed with one-month time shift between them, thus also having slightly different inoculum for each test setup. In total 90 samples were analysed for 6 different feedstock's and two AD temperature conditions.

A volumetric measuring method was used by measuring the biomethane amount through the displacement of a 20 mL syringe piston connected to a batch bottle. For triplicates three best syringes were selected (with lowest friction) and slightly modified (cutting off excess piston rubber to minimize friction). Each syringe was dedicated to specific triplicate in consistent order, thus giving opportunity to see if piston friction changes and affects measurements. To determine the methane concentration without the CO_2 fraction, 5 mL of 3M NaOH alkaline solution was filled into the measuring syringes before each measurement. For extra confidence some of measured samples periodically were left overnight in closed syringes to see if all CO_2 has been absorbed during measurement.

Theoretical BMP according to Buswell's formula

Depending on the type of biomass, the assessment of BMP can eventually require time of up to 90 days (Hansen et al., 2004; Angelidaki et al., 2009; Kafle & Kim, 2013). For a more rapid estimation, a theoretical biomethane potential (BMP_{theo}) can be used from the Buswell equation (Allen et al., 2013) – see formula 1. Once the biomass' chemical compositions of C, H, O are known, it is possible to calculate the BMP_{theo} (Angelidaki & Sanders, 2004) and the correspondent CH_4 fraction as BMP_{theo}.

$$C_nH_aO_b + \left(n - \frac{a}{4} - \frac{b}{2}\right) H_2O \rightarrow \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right) CH_4 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right) CO_2 \qquad (1)$$

where n – carbon atoms in biomass; a – hydrogen atoms in biomass; b – oxygen atoms in biomass.

The methane yield (BMP_{theo}) from the Buswell's equation can be recalculated with a reference to the unit of gram (i.e. g-VS) in standard condition (i.e. STP) (Raposo et al., 2011), see Formula 2.

$$BMP_{theo,yield} = \frac{\left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right) \cdot 22.4}{12n + a + 16b} \cdot \left(STP \frac{lCH_4}{g - VS}\right) \qquad (2)$$

where n – carbon atoms in biomass; a – hydrogen atoms in biomass; b – oxygen atoms in biomass.

Experimental yields are usually lower but knowing the theoretical yield value allows to calculate the efficiency of digestion.

Chemical composition of fish waste fractions was analysed by a Latvian State Institute of Wood Chemistry. Results are presented in Table 2.

RESULTS AND DISCUSSION

Inoculum and substrate characterization

TS and VS content for all three inocula were similar, however, slightly different methanogenic activity was observed referring to the methane volume produced from the blanks (data not shown) and the total accumulated methane amount from samples. Sludge was most active in the second experiment and especially at high temperature conditions. However, that did not have a relevant impact on the final BMP values acquired from batch tests.

Table 2. Chemical composition of different fish waste fractions (for biomass 2)

Substrate	% of TS					
	Carbon (C)	Hydrogen (H)	Oxygen (O)	Nitrogen (N)	Sulphur (S)	Ash
Heads	37.82	4.72	22.51	11.14	0.29	23.51
Skin/bone mix	40.30	5.06	17.37	12.16	0.35	24.75
Intestines	57.17	6.78	12.12	6.17	0.34	17.43
M1	43.55	5.44	19.32	9.53	0.32	21.85
M2	46.89	5.83	16.09	9.64	0.33	21.22
M3	41.51	5.51	20.62	9.77	0.32	22.27

TS and VS content for fish heads and skin/bone mixture (furthermore also referred as ‘skins’) was similar both for the biomass 1 and biomass 2 (Table 1). TS were around 20% and VS were 75–76% of TS. Although homogenized intestine samples seemed more liquid, they showed the highest TS content varying between 36% for biomass 1 and 30% for biomass 2. This could be explained with high lipid content that is not lost during TS drying operation.

Furthermore, this high lipid concentration is affecting BMP test results, showing the highest methane yield for samples with intestines both for high and low temperature conditions. Similar effect was observed by Nges et al., 2012. VS content for round goby’s intestines was similar for both biomass sources reaching 82.6% of TS.

Biochemical methane potential

BMP testing was done with slightly modified 20 mL rubber piston syringes containing 5 mL of 3M NaOH solution for CO_2 absorption. Piston’s friction was constantly monitored and no significant change was detected during all three experiments. Periodically, accumulated gas samples were left overnight in closed syringes to check NaOH solution’s CO_2 absorption efficiency during slow biogas collection. Fortunately, no visible change in gas volume was ever detected. Consequently, the measured biogas values pertain to the methane content produced.

Regarding to **total accumulated** biomethane volume per test vial, significant difference can be seen between low temperature and high temperature batch samples. Overall, for the samples that were incubated at 23 °C an average 23% reduction can be observed in total accumulated biomethane volumes (Fig. 1, A). This matches with trends reported in literature stating that lowering temperature by 10 °C biogas production slows down approximately two times (Seadi et al., 2008; Zhu & Kumar, 2014).

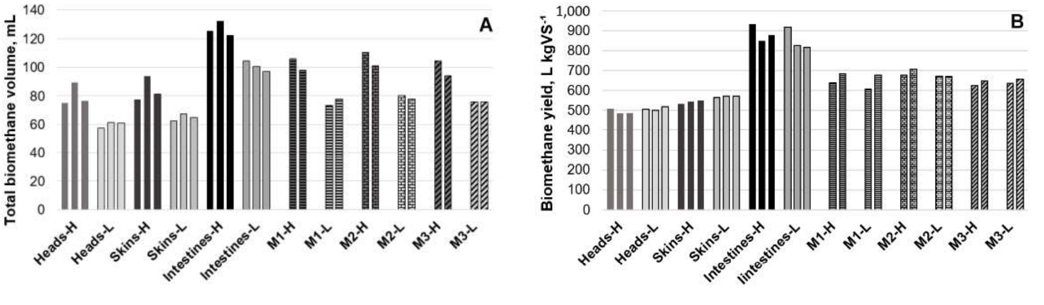

Figure 1. Total accumulated biomethane amount (A) and BMP per 1 kg VS (B) during experiments 1, 2, and 3. Index –H stands for 37 °C, –L stands for 23 °C.

After calculating the net biomethane volumes (by subtracting blank sample volumes from the total accumulated biomethane volumes), the difference between low and high temperature samples occurs to be very low. Furthermore, after calculating the final BMP values (always based on the net biomethane volumes) per kg of VS, the overall average BMP results for low temperature samples are only 2% lower than for 37 °C (Fig. 1, B).

In total, the BMP difference per 1 kg of VS among the two sets of temperature conditions was only 2%. Nevertheless, it must be clarified that the overall difference in total accumulated biomethane amount is 23% (see Fig. 1, A). This result may be due to an extra 23% of total biomethane volume that was contributed by the sewage sludge inoculum at higher temperature. Methanogenic bacteria activity and growth is much lower at low incubation temperature conditions, thus resulting in a slower augmentation and decay (dead biomass methanation) of the microorganism consortium, thereby lowering the amounts of total produced biomethane. This should be taken into account when designing bioreactor for fish waste and sewage sludge co-digestion at low temperature conditions in terms of bigger digester's size. Nevertheless, results of this study suggest that lowered temperature does not have a strong impact on fish waste digestion efficiency and final BMP, however, it affects digestion kinetics.

During all three experiments the highest BMP values were obtained from batch samples containing fish intestines both for high and low temperature conditions (Fig. 1, B). Average biomethane yield from all three experiments at 37 °C was 887 L CH_4 $kgVS^{-1}$ and 853 L CH_4 $kgVS^{-1}$ at 23 °C. These high values are reached because of high lipid and protein content, especially in gonads and fish eggs that were present in Round Goby's abdomens. The theoretical BMP yield for lipids is about 1000 L CH_4 $kgVS^{-1}$, while the theoretical yield for protein is about 490 L CH_4 $kgVS^{-1}$ (Nges et al., 2012). BMP values of first experiment are higher than those of second and third, reaching 933 L CH_4 $kgVS^{-1}$ at 37 °C and 917 L CH_4 $kgVS^{-1}$ at 23 °C. In comparison, results from second and third experiment were only 850–878 L CH_4 $kgVS^{-1}$ for high and 816–826 L CH_4 $kgVS^{-1}$ for low temperature. Despite similar VS content (82.6%) of round goby's both biomasses this difference in results could be explained due to the fact that for first experiment used fish biomass was caught in spring season (April). In spring time fish are ready for new spawning season and have larger gonads and contain more mature fish eggs, thus increasing overall lipid and protein relative share in viscera.

These results are slightly higher than reported 500 L CH_4 $kgVS^{-1}$ for perch (*Perca fluviatilis*) intestines (Tomczak-Wandzel et al., 2013), however, this could be attributed to the fact that relative share of gonads in perch abdomen is much smaller (if present at all in different seasons).

The overall average BMPs acquired from three experiments for fish heads at high temperature and low temperature was 494 L CH_4 $kgVS^{-1}$ and 508 L CH_4 $kgVS^{-1}$ respectively. Skin and bone mix showed slightly higher results, therefore average BMP at 37 °C was 542 L CH_4 $kgVS^{-1}$ but at 23 °C was 570 L CH_4 $kgVS^{-1}$. It can be seen that at lower temperatures average BMP values are slightly higher than at 37 °C both for heads and skin/bone mixture. This could be explained due to the fact that for several high temperature samples after 20 days' biomethane production was delayed and a slight inhibition of methane production was observable, as blank reference samples on daily basis produced more gas than samples containing fish waste. This in fact resulted in negative daily net biomethane values, indicating the start of inhibition which is consequential after digestion of high organic content substrates and rapid VFA accumulation, as can be observed also during dairy product anaerobic digestion (Labatut et al., 2011). This also is in line with literature where it is suggested that AD under lower temperature conditions is more stable and less volatile fatty acids are accumulated

(Appels et al., 2008). However, no great change in pH was observed at the end of all experiments, only for few samples lowering from pH8 to pH 7.7.

Summary of BMP values acquired during this research for different fish waste samples can be seen in Table 3.

Table 3. Summary of estimated yields from Buswell's equation and experimental CH_4 yields

Substrate	BMP_{theo} (L CH_4 kgVS^{-1})	BMP at 37 °C (L CH_4 kgVS^{-1})	BMP at 23 °C (L CH_4 kgVS^{-1})
Heads[1]	–	509.2 ± 29.5	506.3 ± 1.0
Skin/bone mix[1]	–	533.0 ± 17.8	565.4 ± 110.8
Intestines[1]	–	933.1 ± 60.9	916.9 ± 39.7
Heads[2]	625.0	485.4 ± 20.2	500.8 ± 14.9
Skin/bone mix[2]	728.9	544.9 ± 25.5	572.6 ± 26.3
Intestines[2]	895.7	849.8 ± 15.4	826.1 ± 26.0
M1[2]	719.4	639.1 ± 4.8	609.2 ± 11.6
M2[2]	791.8	677.6 ± 18.0	672.4 ± 11.0
M3[2]	769.0	626.3 ± 24.5	636.7 ± 2.5
Heads[3]	625.0	488.8 ± 18.6	519.6 ± 19.1
Skin/bone mix[3]	728.9	548.8 ± 24.4	572.2 ± 22.9
Intestines[3]	895.7	877.7 ± 41.8	816.3 ± 51.9
M1[3]	719.4	685.7 ± 17.4	676.5 ± 27.0
M2[3]	791.8	709.2 ± 37.5	668.6 ± 30.7
M3[3]	769.0	649.5 ± 10.3	657.6 ± 18.4

[1] – experiment 1 (biomass 1); [2] – experiment 2 (biomass 2); 3 – experiment 3 (biomass 2).

Three different fish waste fraction mixes were also prepared. First mix (M1) contained all waste fractions in equal share based on TS. Second mix (M2) contained all waste fractions in equal share based on wet weight. Third mix (M3) contained all waste fractions in wet weight ratios: 2 parts heads, 2 parts skin/bone mixture, 1part intestines (based on practical fish processing approach). M1 average BMP at 37 °C and 23 °C was 662 L CH_4 kgVS^{-1} and 642 L CH_4 kgVS^{-1} respectively. M2 average BMP at high temperature was 693 L CH_4 kgVS^{-1} and 670 L CH_4 kgVS^{-1} at low temperature. M3 average BMP at high temperature was 638 L CH_4 kgVS^{-1} and 647 L CH_4 kgVS^{-1} at 23 °C. No significant difference can be seen regarding to anaerobic digestion of these three mixes, thus any of these three compositions can be successfully used for biomethane production. As expected, average BMP was around 660 L CH_4 kgVS^{-1}, that is similar to mathematical average from heads, skins and intestines BMPs'. Other authors report similar results for Pacific saury, Nile perch, mackerel and cuttlefish wastes, ranging between 562–777 L CH_4 kgVS^{-1} (Kassuwi et al., 2012; Kafle et al., 2013). BMP for cod meat and intestine mix was reported to be 503–533 L CH_4 kgVS^{-1} after 14 days long incubation period (Almkvist, 2012; Shi, 2012). Regarding to 14-day period BMP from round goby's waste mix is slightly higher reaching approximately 640 L CH_4 kgVS^{-1}. In this light, it would be advisable to measure BMP for more extended time period, as far as it is reasonable, to obtain fully total BMP of biomass.

Dynamics of biomethane production

Cumulative curves and dynamics of biomethane production are shown in Fig. 2. For high temperature samples the main production was observed during the first 7–9 days, accounting for 95% of the total BMP. In turn for low temperature conditions main biomethane production was observed during first 14–16 days, accounting for 94% of the total BMP.

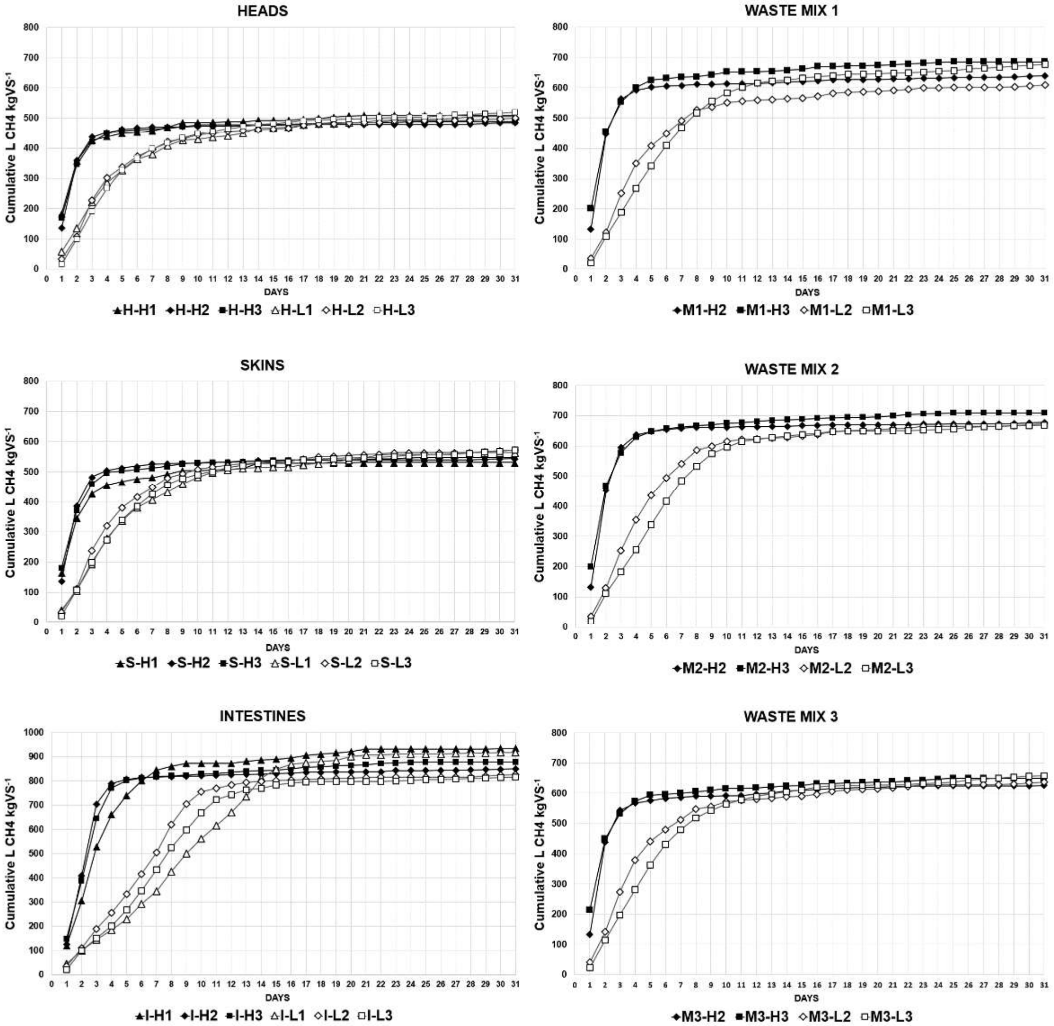

Figure 2. Averaged triplicate methane production dynamics trough experiments 1, 2, and 3. Indexes –H stands for 37 °C; –L stands for 23 °C; 1, 2, 3 stands for experiments 1, 2, 3.

Similar pattern regarding to fish waste highest production rate time shift was reported by (Chen et al., 2010), where highest biogas production rate under thermophilic conditions (50 °C) was achieved on day 10, in comparison to 17 days at mesophilic (35 °C) conditions. Moreover, this great difference could be also attributed to type of inoculum that was used in this research, because sewage sludge was gathered from bioreactors that normally operate at 37 °C. Shift to low temperature conditions put extra stress on microorganism consortium. It is also suggested that more appropriate microbial consortium can be developed and adapted for fish waste AD by sequential addition of fish based feedstock, thus making optimized inoculum for substrates with low C:N ratios (Quinn et al., 2016).

Nevertheless, slower biomethane production rate had no significant impact on final BMP results. In addition, slower digestion time means that substrate needs longer hydraulic retention time (HRT) in digester (Dhaked et al., 2010; Zhu et al., 2014), thus slowing down biogas production or forcing to increase digesters size. On average, lowering fermentation temperature by 10 °C required anaerobic digester's size increases 2–2.5 times (Balasubramaniyam et al., 2008). However, digester's size can be reduced if shorter HRT is selected. In respect to this research results, it would be more reasonable to use a HRT of 15 days instead of 30 days for low temperature fish waste anaerobic digestion, as more than 94% of BMP is achieved during this short time.

CONCLUSIONS

The results of this research show that AD of round goby's processing waste at 23 °C is twice as slow as under 37 °C conditions. Thus prolonging hydraulic retention time (HRT) needed for complete biomethanation of feedstock, in turn increasing necessary size of digester. However, costs of digesters size increase should be compared to savings on insulation materials and heat energy input. Thus most feasible approach regarding to ratios of digesters size, HRT and fermentation temperature could be found.

For low temperature conditions an overall 23% reduction in total produced biomethane volume was observed. However, this difference is attributable to the inoculums specific activity at different temperatures and counteracting the contribution to the total biomethane volume, rather than to feedstock's biomethanation efficiency. Despite the fact, that several fish waste fractions such as heads and skins showed higher BMP values at lower temperature, based on overall averaged results, in general only a 2% reduction in total BMP outcome was observed for low temperature samples after 25 days, thus showing that biomethanation is still efficient also at lowered temperatures.

Round goby's processing wastes could be successfully used for biogas production in co-digestion, especially if containing intestines, however in-depth research is still needed to find out possible inhibitory effects and mechanisms. Also volatile fatty acid accumulation and inhibitory effect during continuous low temperature fermentation should be researched. Furthermore, AD of *Neogobius melanostomus* under psychrophilic conditions should be explored.

Research results suggests that anaerobic digestion of fish waste under low temperature conditions could be feasible as the process still occurs with 98% efficiency in respect to 37^{0}C, in fact opening a new opportunity to explore the overall sustainability of technologies based on these conversion processes.

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