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THE USE OF OUT-OF-DATE FROZEN FOOD AS A SUBSTRATE FOR BIOGAS IN ANAEROBIC METHANE FERMENTATION

Abstract
The declining quantity of fossil fuels and the increasing demand for energy are two of the many problems that are affecting current times. This is why there is an increasing interest in and introduction of operations related to renewable sources of energy. One of many such possibilities is to conduct the biomass methane fermentation process and to obtain clean energy in the form of biogas. The aim of this research was to analyse the biogas-generating potential (Biochemical Methane Potential) of out-of-date frozen products in the laboratory anaerobic fermentation process and the near-infrared method with appropriate Biochemical Methane Potential calibration. The results obtained showed that selected frozen products are an excellent material for use as substrates resulting in the production of high quality biogas. It gives the opportunity to continue research, mainly in terms of applications, e.g. for biogas plants, using other available products on the market and the selection of their mixtures.

Key words
renewable source of energy, biogas, methane fermentation, methane, inoculum, BMP, out-of-date frozen food

Introduction

With the advancement of civilization and the economy, the need for everyday comfort and the satisfaction of the physiological needs of people is growing, among which, above all, food access can be distinguished. This is conditioned by many factors, including the degree of advancement of agriculture or food processing. New sales markets are created and quick and easy access to the purchase of goods, and fairly new food products are introduced onto the market, not always to the satisfaction of all consumers. In developed countries, there is a significant overproduction of food in relation to actual demand (domination of supply over demand), and, at the same time, an increasing interest in the diversity of the food products made available by competing producers. This leads to the wasting of excess quantities of food that could be used for consumer purposes. This food becomes a potential waste, so it is worth looking for methods of its effective use [1, 2]. Every year, according to the Federation of Polish Food Banks, nearly 1.3 billion tons of food is wasted, which is about 1/3 of the total food produced (Europe: approx. 100 million tons, Poland: approx. 9 million tons, of which about 6.6 million tons comes from the food industry) [3]. Waste food is nothing more than a simultaneous waste of energy and water at the stage of production, transport and further management, and also leads to the creation of more waste [4]. This is due to many reasons: a lack of accurate estimation of production volume, damage to the production line, changing consumer preferences, inadequate food storage, packaging defects, natural disasters and many other causes, the most frequent being out-of- food products [1; 2].

Nowadays, so-called "Convenience food", mainly due to the seasonality of some raw materials or the ease of storage are very popular. This is aimed at giving products a greater availability by processing which prolongs their shelf live. One method of food preservation is the low temperature treatment: the cooling and freezing of food. While cooling extends the shelf life of products, this time is comparatively short. Freezing of products ensures a long shelf life with no loss of valuable ingredients. Thus, vegetables and fruits are very popular when subjected to freezing on a mass scale [5, 6, 7]. The problems mentioned above concerning the quality and durability of products lead to the generation of food or waste biomass in huge quantities. Not only are people obliged to optimize the production conditions, but also to develop methods and technologies that will facilitate the re-use of waste biomass and at the same time to reduce its negative impact on the environment [8].

Biomass energy is one of the most popular renewable energy sources, because, among other reasons of its availability. The energy stored inside is transformed into different forms of biofuels: solid, liquid and gaseous. One of the perfect conversion methods seems to be the methane fermentation process in which organic

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multiparticulates are broken down then biodegraded by microorganisms, so that in the end, one of the valuable energy carriers, i.e. biogas, is obtained [9, 10]. It consists primarily of methane (40-75%) and carbon dioxide (25-60%), as well as other gases present in small amounts such as nitrogen, hydrogen sulphide, oxygen, hydrogen, carbon monoxide and others. CH_4 in and of itself is a high-energy gas, and its content shows the quality of the biogas - the greater its share of biogas, the higher the fuel's heat of combustion. This composition depends on the conditions of the fermentation process [11, 12]. Ensuring optimal process conditions is the key to achieving the most satisfying results. Among the factors that can be distinguished are: pH, temperature, particle size, mixing, nutrient concentration, substrate moisture and inhibitors, as well as the proper selection of substrates or inoculum [13]. Special attention should be given to nature of substrates, including the right choice of substrates, the way they are prepared for the process (size, dry or wet material), retention time and loading rate. These factors affect the digestion process efficacy and the same gas production rate [14]. The optimal pH range for efficient anaerobic digestion is 6.8-7.2. Lower pH lead to reduce growth rate of methanogens, in turn higher value of pH (alkaline) have influence on disintegration of microbial flux [15]. Temperature affects the nature of microorganism metabolism, biomass composition, dietary requirements and reaction speed. For suitable groups of organisms, the increase in temperature affects the rate of growth, and too high a temperature (above the optimal value) causes protein denaturation [16]. The optimum mixing ensures maximum biogas yield by ensuring better contact of bacteria with substrate particles. On the other hand, too intensive mixing can cause foaming, sedimentation, frothing materials and scumming [17]. In addition, the C / N ratio is often specified. Its inadequate value may release of high volatile fatty acids accumulation, high total ammonical nitrogen or free ammonia. Many years of research indicate that the most optimal range for bacterial growth is 20-30 and it is variable depending on the introduced substrate [15]. The industrial scale process itself has to be optimized to increase the production of biogas and methane at the same time. Biochemical Methane Potential tests are becoming more and more popular, because they characterise a given substrate before it is introduced into the fermentation chamber. It indicates what amount of methane can be potentially produced from a given substrate. It measures the maximum amount of biogas or methane produced per gram of volatile solids ($cm^3_{CH4} \cdot g_{VS}^{-1}$), contained in the organics used as substrates in anaerobic methane fermentation. BMP can measure the methane potential of different substrates as well as pure, individual products and their mixtures. There are a few experimental methods available but BMP analysis is the most successful, thanks to their easy setup, conduction and the useful information obtainable from them for optimizing the design or operation of an anaerobic digester [18, 19]. The benefits from methane fermentation lead to an increase in interest in the use of this technology. Correct system design and precise planning are really necessary to maximize efficiency. Biochemical methane potential is relatively cheap, repeatable and does not require heavy labour and provides a real appraisal of the anaerobic digestibility of the substrate [20].

The presented paper displays the results obtained in the laboratory BMP tests of the mesophilic periodic methane fermentation of expired frozen vegetables under different organic loads and with the use of two different inoculums. Moreover, experimental results are compared with Near Infrared Spectroscopy (NIR) analyses with an appropriate empirical calibration delivered by the NIRFlex N-500 SOLID apparatus (Buchi). The CHNS analysis of substrates made it possible to relate the C/N quotient of each substrates to its methane production potential. The biogas production efficiency was strongly influenced by the inoculum type, which suggests that microorganisms of methane fermentation need to be seasoned in order to use the substrates efficiently. The anaerobic fermentation of the examined out-of-date frozen products lead to the production of biogas characterized by its high methane content which makes them a very attractive substrate for biogas plants.

Materials and methods

The biogas-generating potential was investigated for four selected out-of-date frozen products, i.e. carrot, green peas, yellow beans and mixed vegetables (composition: 35% carrots, 25% green peas, 20% corn, 10% celery, parsley 10%). At the same, time two types of inoculum were used: fermented sewage sludge, taken directly from the installation of mesophilic methane fermentation located in the Wastewater Treatment Plant in Lodz (WTP; process temperature about 37°C); digestate from agricultural and utilisation biogas plant (BP) (mainly substrates: apple pomace, decoction and waste from the food industry, process temperature 44°C).

In order to determine the suitability of frozen vegetables as methane fermentation substrate, the C/N ratio was analysed by elemental composition analysis (CHNS) with CE Instruments, the NA-2500 apparatus. The above-mentioned products were defrosted and pre-treated mechanically – only by milling. The prepared substrates were added to the fermentation mixture (without drying). Process conditions: one-stage, periodic wet fermentation with periodic mixing, carried out in flasks with a working capacity of 500 cm^3 in thermostated incubators equipped with a biogas volume measurement system (process temperature 37-40°C). The following load of wet substrates: 20, 30 and 40 $g{\cdot}dm^{-3}$ and zero tests (the inoculum itself) were investigated, it corresponds to the volatile solids (VS) concentration of individual vegetables in the fermentation mixture (in $g_{VS}{\cdot}dm^{-3}$): carrot (2,00; 3,00; 3,99), mixed vegetables (3,02; 4,53; 6,03), green peas (5,05; 7,58; 10,10), yellow bean (2,61; 3,91; 5,22). The composition of the produced biogas was measured with gas chromatography for CH_4 content (gas chromatograph SRI Instruments, 8610C Compact GC). The pH measurement in fermentation mixtures was carried out with WTW SenTix41 pH electrode. The results obtained of biogas production efficiency were compared to Biochemical Methane Potential (BMP) analyses with a near-infrared spectrometer (NIR FLEX N-500 SOLID). The BMP was assessed by near-infrared spectroscopy (BMPNIR) combined with rapid and innovative calibration (FlashBMP®) developed by Ondalys (Chemometrics – Data Analytics) and commercialized by Buchi (Switzerland) [21, 22]. The results of measurement are given in cm^3 of methane per g of VS. As to experimental the total volume of biogas was measured than background volume was subtracted. Then on the basis of methane concentration in biogas the total amount of methane produced was calculated and divided by volatile solids added with a substrate. That gives results in cm^3 of methane per g of VS.

Results and discussion

As the first part of the research, differences in biogas production from the inoculum were checked (Fig. 1). There is a significant difference between these sludges, the almost five-fold greater biogas production (WTP: 235 cm^3, BP: 1220 cm^3) was observed, furthermore the biogas BP sludge was produced for a longer period. This difference is directly related to the intake site, i.e. the WTP sludge was collected from the sludge degassing tanks after the fermentation process, and the other directly collected from the fermentation chamber. The obtained volume of biogas from the inoculum was subtracted from the biogas volume produced in the BMP tests which was the basis for checking which type of inoculum would prove to be more effective in testing – under the same process conditions.

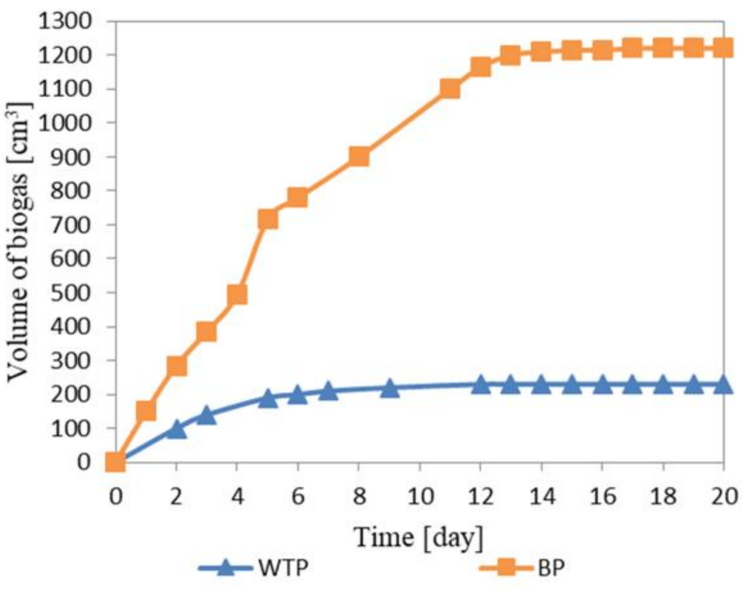

Fig. 1. Biogas production from two inoculums: 1) Wastewater Treatment Plant (WTP); 2) biogas plant (BP)
Source: Author's

The use of out-of-date frozen products as substrates gave positive results taking into account the quantity and quality of biogas produced. Figure 2 shows examples of process results for trials with a load of 40 $g{\cdot}dm^{-3}$. The most intensive production was observed up to 5-6 days. Biogas production on the WTP sludge lasted a maximum of 12 days, while on the inoculum from BP, it stopped after 17-20 days. Biogas production was slightly more efficient in the fermentation mixtures with inoculum from BP.

The BP sludge more effectively used the added substrate (carrot, mixed vegetables) and, as a result, larger volumes of biogas were obtained from the same loads of the tested vegetables (Figure 3). Therefore, the fermentation process for peas and beans was continued only on this sludge. The selection of an appropriate inoculum as well as the acclimatization of sludge to the used substrate plays an important role in the methane fermentation process, this fact is confirmed in the paper by Yin et al. dealing with volatile fatty acid production from food waste in the methane fermentation [23]. On the other hand, Koch et al. suggest that the choice of inoculum had no significant impact on the specific methane yield of the tested substrates. They also suggest that the degradation is faster for those inocula already adapted to the substrate tested while efficiency stays the same [24].

In terms of the amount of obtained biogas, peas turned out to be the best of the tested vegetables (max 2945 cm^3). The organic load of the substrates is another factor influencing the process (Figure 3), with each increase a larger biogas production was noted. However, these were not the highest VS loads that could be used, the processes ran in the stable conditions, where the initial pH was within in the range of 7.54-7.76; and the final 7.34-7.57 (optimal, comparable level). Czerwińska and Kalinowska suggest that the optimal range is between 4.5-7.5 (6.8-7.2 for the methanogenic phase), but the results are only slightly out of range. With an excessive amount of VS, the process could be disturbed or completely stopped due to volatile fatty acid accumulation [13]. Sosnowski et al. reports that during fermentation an accumulation of volatile fatty acids (VFA) caused a decrease in pH and strongly inhibited gas production. The more optimal the conditions are provided for bacteria, the higher the percentage share of methane in biogas can be obtained [25].

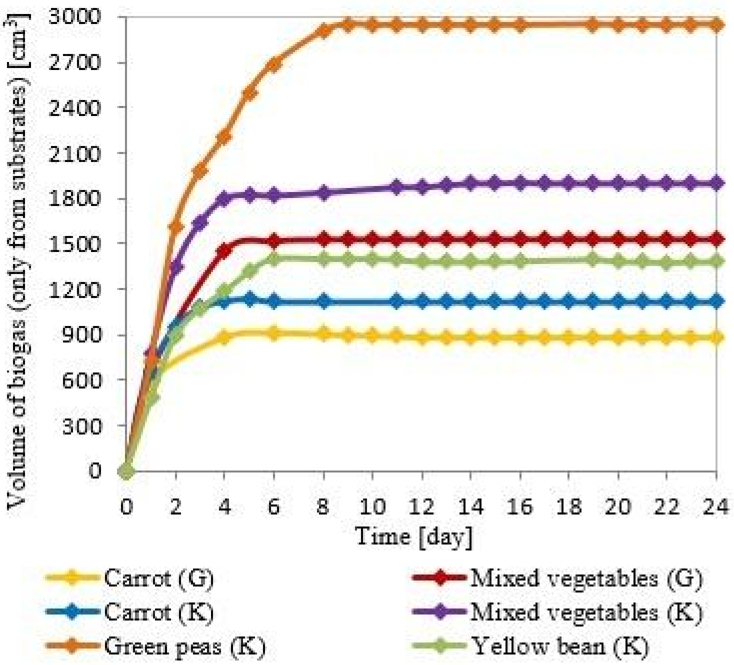

Fig. 2. Biogas production from out-of-date frozen food without inoculum. Fermentation mixtures with a load of 40 $g \cdot dm^{-3}$ (K – BP, G – WTP)

Source: Author's

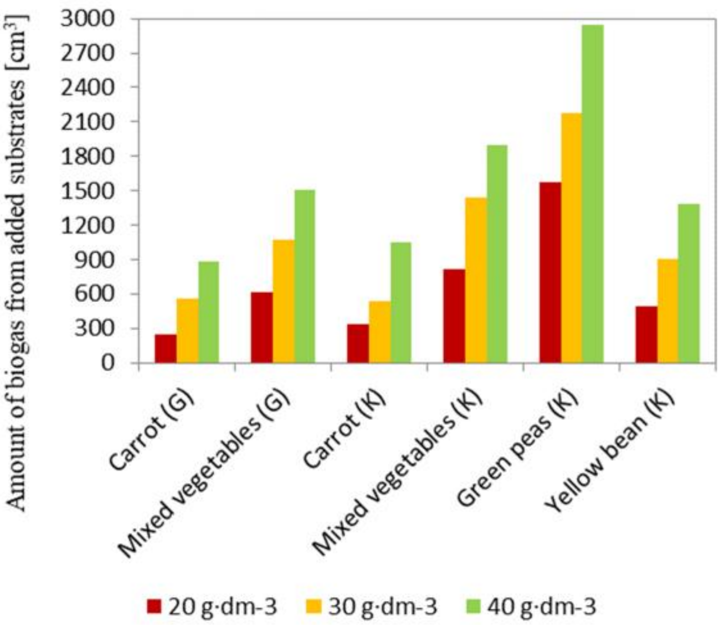

Fig. 3. Volume of biogas only from out-of-date frozen food (without inoculum); Fermentation mixtures with a load in all cases (K – BP, G – WTP)
Source: Author's

Table 1 shows the percentages of CH_4 from the individual fermentation series. In terms of quality (averaging results), the biogas plant sludge was also more attractive with a methane content of 68.05-70.61%, although these values are comparable. The addition of substrate contributed to the enrichment in methane by about 0.2 to 18.6%. Such a high share of the most important component (from 63.98 to 78.18%) indicates the possibility of obtaining high-quality biogas from frozen food. Jędrczak presented the range of methane content in biogas between 40-75%, while Kaźmierowicz in presenting all chemical components of agricultural biogas, shows that methane can be in the range of 52-85% [26, 12]. Comparing the obtained results, it is stated that they belong to the upper range.

The optimal value of the C/N ratio of substrates, indicated by many literature positions, is in the range of 10-30, variable depending on the source. Głodek, as the optimal one, gives exactly the above scope (10-30), in turn Czerwińska and Kalinowska and Jędrczak reduce this range, i.e. 10-25. Deubelin and Steinhauser, on the other hand, state that it should be in the range of 16-25 [28, 13, 12, 27]. Figure 4 shows the methane yield in relation to the C/N ratio for each of the tested substrates. The carbon-to-nitrogen ratio of all examined substrates is in the above mentioned range, once near the highest values - carrot (29.32) or the lowest as in the case of green peas (10.5). However, by comparing the results to the limits presented by Deubelin and Steinhauser in 2011, only the vegetable mix (17.98) would comply with the requirement [27]. The type of substrate used also affects the stability and efficiency of the process. There are products on the market with a diverse elemental composition (and the same C/N ratio) and they also differ in the DS and VS content. It is worth looking for products that will most effectively affect the fermentation process - not only individual substrates, but also all kinds of mixtures. Both mixtures of substrates as well as the widely understood co-fermentation seem to be a more effective solution. The paper by Sadecka et al. shows the many advantages of co-fermentation: the possibility of five times more biogas production, a higher degree of VS decomposition and less contamination of digestate suspensions [29]. Co-fermentation is more favourable than mono-fermentation also according to Sosnowski et al., which additionally draws attention to the benefits in terms of energy saving and environmental protection [25].

Table 1. Range of methane share in all series

Substrates	Inoculum	Methane share [%]
Carrot	WTP	63,98 - 75,16
Mixed vegetables		65,85 - 78,18
Carrot	BP	69,59 - 73,82
Mixed vegetables		67,24 - 68,25
Green peas		74,26 - 74,84
Yellow beans		73,06 - 73,10
Sludge from WTP		63,62 - 72,08
Sludge from Konopnica (BP)		68,05 - 70,61

Source: Author's

However, the study showed that the green peas with the lowest C/N ratio (Figure 4) , close to the lowest range indicated above, was the most efficient substrate in terms of methane production per 1 gram of VS delivering close to 450 $cm^3_{CH4}\cdot g_{VS}^{-1}$. Whereas the carrot, having a C/N ratio almost going above the optimal ranges, was characterized by the lowest methane production, 276 $cm^3_{CH4}\cdot g_{VS}^{-1}$. Nevertheless, the direct correlation between C/N ratio and methane yield was not found. Dioha et al. show in the article, the correlation of biogas yields of tested substrates with C/N ratios, based on the part of waste such as rice husks, sugar, bagasse, grass silage, animals excreta. In fact, for the range of C/N for the substrates above 30 and from the lower range 10, much smaller volumes of biogas were obtained (for C/N: 47, 53, 82, 10; respectively: 280, 200, 150, 28 $cm^3\cdot g_{VS}^{-1}$). In turn, the higher production of biogas was characterized by the substrates with C/N: 26; 25; 24; 15; 13 (biogas: 650, 350, 700, 350, 500 $cm^3\cdot g_{VS}^{-1}$). However, the results of the obtained biogas in these ranges are divergent [30]. As was shown there is no optimal universal C/N ratio for methane fermentation It has the effect on methane production and in the case of too low a value, can result in ammonium inhibition and if too high the nitrogen supply can be not enough to satisfy the metabolic needs of microorganisms. since the C/N ratio cannot be the basis on which the substrate composition can be optimized.

The laboratory BMP tests may not be extremely laborious and the obtained results the most reliable ones but nevertheless they require a lot of time. Utilisation of out-of-date food results in the unstable substrate supply in terms of composition and diversity. The near-infrared spectra analysis with appropriate calibration can be useful for predicting methane yields in a matter of minutes and can potentially be implemented as an online measurement as suggested by Ward [31]. According to Preys S. et al. a long-term series of laboratory tests are indispensable to obtain the proper calibration curve of NIR results and Biochemical Methane Potential [18]. Nevertheless, accurate and approved calibrations are available on the market (i.e. https://www.buchi.com/en/products/ nirsolutions/nirflex-n-500).

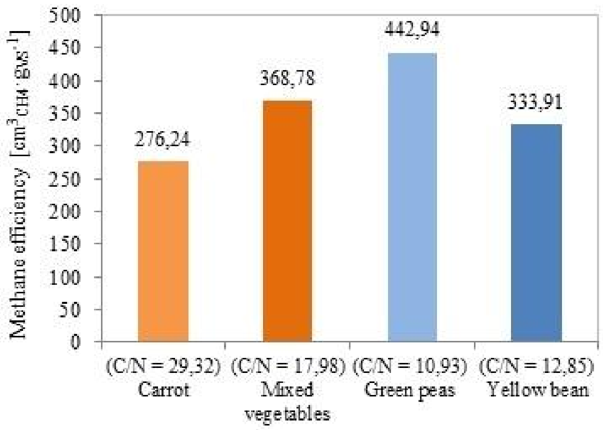

Fig. 4. Methane efficiency in relation to the C/N quotient of each substrates – taking into account the results of all series
Source: Author's

Table 2 presents the results obtained from the NIR analysis returned as cm^3 CH_4 per gram of VS delivered with a substrate. The obtained values were compared with the averaged results of the experimental BMP process (also presented in Table 2).

Similar values were recorded for the vegetable mix and yellow bean. The results obtained from the fermentation process for green peas (442.94 $cm^3_{CH4}\cdot g_{VS}^{-1}$) outperformed the significantly predicted value from NIR analysis. Unfortunately, the predicted result for carrots was not obtained in the experiment, and the difference was about 80 $cm^3_{CH4}\cdot g_{VS}^{-1}$. Nevertheless, the investigated organic loads were not the highest ones and these results are valuable information for the calibration and validation of processes. Esposito G. et al. draw attention to the fact that such tests are relatively inexpensive, easy to perform and repeatable, despite the criticisms they face [19].

Table 2. Comparison NIR analysis BMP with results of experiments in all series (methane fermentation)

Substrates	BMP (NIR analysis)	Inoculum	Amount of methane per VS added (methane fermentation)	
			Experimental results	Average value
	$cm^3_{CH4}\cdot g_{VS}^{-1}$		$cm^3_{CH4}\cdot g_{VS}^{-1}$	$cm^3_{CH4}\cdot g_{VS}^{-1}$
Carrot	**356,85**	WTP	264,08	**276,24**
		BP	288,4	
Mixed Vegetables	**353,48**	WTP	329,29	**368,78**
		BP	408,27	
Green Peas	**373,34**	BP	442,94	**442,94**
Yellow bean	**300,48**	BP	333,91	**333,91**

Source: Author's

Summary and conclusions

The obtained results indicate that out-of-date frozen food is a good material for use as a substrate in the methane fermentation process. It is worth noting that the presented values represented only four selected products (carrots, green peas, yellow beans and mixed vegetables) from among the many available on the market. Each of them will have a different influence on the course and final result of the process. The advantage of using such products is that they do not require complicated pre-treatment. In the final effect, one can get high-methane biogas, while reducing the waste from the food sector.

This type of research is of an application nature, however, such materials are used in biogas plants in much larger volumes. The biogas production efficiency will be influenced by such factors as: type and concentration of materials in the digester, pre-treatment, the type of inoculum and others.

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