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0273-2289/03/109/0107/$20.00

Reducing Waste Contamination from Animal-Processing Plants by Anaerobic Thermophilic Fermentation and by Flesh Fly Digestion

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Abstract

There is currently no market in Israel for the large amounts of waste from fish- and poultry-processing plants. Therefore, this waste is incinerated, as part of the measures to prevent the spread of pathogens. Anaerobic methanogenic thermophilic fermentation (AMTF) of wastes from the cattle-slaughtering industry was examined previously, as an effective system to treat pathogenic bacteria, and in this article, we discuss a combined method of digestion by thermophilic anaerobic bacteria and by flesh flies, as a means of waste treatment. The AMTF process was applied to the wastes on a laboratory scale, and digestion by rearing of flesh fly (*Phaenicia sericata*) and housefly (*Musca domestica*) larvae on the untreated raw material was done on a small scale and showed remarkable weight conversion to larvae. The yield from degradation of poultry waste by flesh fly was 22.47% (SD = 3.89) and that from fish waste degradation was 35.34% (SD = 12.42), which is significantly higher than that from rearing houseflies on a regular rearing medium. Bacterial contents before and after thermophilic anaerobic digestion, as well as the changes in the chemical composition of the components during the rearing of larvae, were also examined.

Index Entries: Thermophilic; anaerobic methanogenic thermophilic fermentation; fly larvae; poultry processing; fish processing; pathogenic bacteria.

Introduction

Because of the long history of concern about serious health and safety issues, including the treatment of the various waste streams from processing plants, we decided to address the health hazards arising from the fish- and poultry-processing industries. The poultry industry can be

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divided into two stages, each with its own particular hazards. The first stage comprises the raising of live birds to the desired weight, their delivery to the processing plant, and preparation for slaughter. The second stage comprises the slaughtering, processing, and packaging of the birds. A potentially significant hazard from the first stage arises from the presence of microorganisms and endotoxins in the organic wastes, and an effective health and safety program for the slaughtering stage must also include control of the waste stream. Most of the wastewater emitted by the poultry industry comes from the slaughtering process, in which the birds are bled, scalded with hot water, rinsed up to three times, gutted, and chilled with water. The needs both to maintain high production and to meet zero fecal contamination requirements make the use of large volumes of water inevitable. On-site treatment of the wastewater, before its discharge from the plant, is the usual practice today, to prevent poultry processing byproducts from entering and contaminating the sewage system outside the factory. The solid fraction is rarely used and is incinerated. Organic solid waste from the processing operations comprises rinds and seeds from the feed, and skin and bones from the birds. Similarly, in the fish-processing and -packaging process, a large amount of waste is produced.

We present an integrated way to use all the wastes from both the poultry- and fish-processing processes, by treating them by means of anaerobic methanogenic thermophilic fermentation (AMTF) and by breeding fly larvae, which digest the resulting slurry. Figure 1 illustrates the integrated approach.

Waste products of slaughterhouses, such as the animals' intestinal contents, blood, urine, and feces, have been found to be highly contaminated with pathogenic microorganisms and also to increase the chemical oxygen demand (COD) of the sewage considerably. AMTF was previously examined as an effective system to eliminate pathogenic bacteria such as *Salmonella* and coliform; it was found to reduce COD and simultaneously to generate energy in the form of methane gas *(1)*.

The use of fly larvae as a means of utilizing organic wastes is an innovative approach that is also relevant to current environmental problems. The great capability of fly larvae to digest organic wastes stems from their biologic characteristics, including their capability to multiply rapidly; the housefly lays approx 100–150 eggs in every clutch and can do so every 2–4 d. One kilogram of cow dung is sufficient for the development of up to 1500 fly larvae. At 25°C the transformation of a larva to an adult housefly can take 5 to 6 d, during which the biomass (wet wt) of larvae, which can increase 100-fold in 2–4 d, can reach 40% of the wet wt of the wastes, leaving an inorganic residue that can be used as an effective fertilizer *(2)*.

The larvae produced could be used as a resource for applications that are yet to be defined, but that could include chick or fish feed, or medicinal uses. In the medical field, fly larvae therapy exploits live larvae to clean intractable wounds; since the mid-1990s, it has been reintroduced for this purpose, and the literature on larvae therapy is rapidly expanding *(3)*.

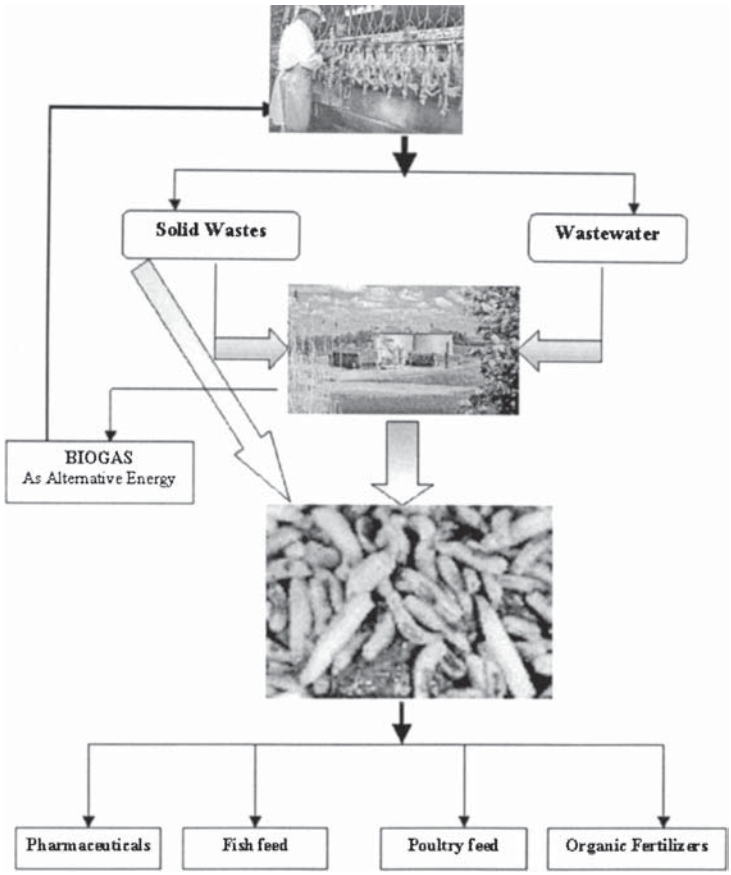

Fig. 1. Schematic description of proposed integrated project.

Medicinal larvae are recognized to have beneficial effects on wounds; debridement, or elimination of necrotic tissue, is one mechanism by which wounds are cleaned, and secreted enzymes have also been described *(4,5)*. Growth-stimulating effects of *Phaenicia sericata* larvae extracts on human fibroblasts have been described, and have been hypothesized to be the basis of one of the mechanisms of wound healing by maggots *(3)*.

Compared with other industries, the food-processing industry is not considered energy intensive. Facilities usually use electrical power, obtained from local utilities, to run food-processing machinery, but the use of fossil fuel is low. A secondary benefit of the AMTF process will be the availability of the biogas it produces for generating electricity in cogeneration systems, which will also produce hot water for use in the process.

Materials and Methods

Wastes

Solid waste—i.e., residues from the poultry-deboning process, usually called "dog food"—from the Off-Hagalil poultry-processing plant was

used. This material includes crushed bones with a small amount of poultry meat. Also used was solid waste from the Yona Tuna and Sardines fish-processing factory in Tirat-Hacarmel; it comprises residues from tuna processing and packing.

In the materials, as received from the processing plants, the concentrations of solids in the poultry and fish wastes were 55.92 ± 2.12 and 26.25 ± 0.24%, respectively. Analysis of proteins, carbohydrates, lipids, and ash contents are presented in Tables 3 and 4. There were problems of heterogeneity of the materials, but this was a result of the conditions and processes in the plants. After its delivery from the plants, the material was kept at –20°C until needed; it was then used "as is" in the experiments on growing fly larvae, or mixed with regular water, to 15% solids content, for feeding into the digestion system.

*Rearing Houseflies (*Musca domestica*)*

Houseflies of the Bet Dagan strain were reared in the insectary in 80% relative humidity, at 26–28°C, and during a 12-h light/12-h dark cycle. Adults were maintained in cages measuring 50 × 50 × 50 cm and fed on granular sugar into which a small quantity of liver powder and yeast extract were mixed. Water was offered in a beaker with floating polyester flakes to prevent drowning of the flies. Into each cage of flies was inserted a large Petri dish containing wet cotton wool smeared with milk powder, to serve as an oviposition medium. After eggs were laid, they were transferred to vessels containing a wet mixture of 9:1 bran and milk powder, respectively. To this standard mixture various proportions of animal waste were added.

The life history of the houseflies was as follows: to oviposition, 2 to 3 d; from oviposition to emergence, 1 d; from larval stages to pupation, 5 to 6 d; and from pupa to cleaving of adults, 2 to 3 d.

*Rearing Flesh Flies (*P. sericata*)*

Flesh flies of the Bet Dagan strain were reared in the same type of cages under the same environmental conditions and were provided with sugar and water in the same way as the houseflies. In addition, two to three times per week a large Petri dish containing liver was offered during the night, to serve for oviposition. The following morning eggs were removed from the liver and placed on animal waste that was laid on a piece of aluminum foil, which was then put into a container, on top of a layer of wood chips that filled one-third of the container volume. The lid of the container was fitted with a net screen to permit aeration while preventing escape of the larvae. Grown larvae of the third instar left the waste and burrowed into the wood chips to pupate. The pupae were then separated and transferred to new cages for emergence. The life history of the flesh flies was as follows: to oviposition, 2 to 3 d; from oviposition to emergence, 1 d; from larval stages to pupation, 5 to 6 d; and from pupation to cleaving of adults, 2 to 3 d.

The grown third-instar larvae were separated from the wood chips with a sieve prior to pupation, and the larvae obtained were kept in a

refrigerator at –20°C pending chemical or microbiologic examination, or were weighed to determine the biomass growth.

The larval chemical composition (protein, fat, minerals, dry weight) was analyzed, and the optimal concentration of eggs in the growth medium was studied. The chemical composition of the growth medium before and after removal of larvae was analyzed, to determine the percentage of undigested material.

Chemical Analyses

Proteins, lipids, minerals, water, and dry matter content were determined by standard methods as specified by the Association of Official Analytical Chemists *(6)*.

Bacteriologic Analyses

Total aerobic bacterial counts were done on 10-g samples that were removed aseptically and homogenized in a Stomacher 400 (Model BA6021; Seward, UAC House, UK) for 1 min in 90 mL of sterile peptone (0.1% [w/v]). Tenfold serial dilutions of the same diluting medium were plated on plate-count agar (Difco) and were then incubated at 30°C for 48 h. The 20 dominant colonies from each plate were restreaked onto nutrient agar and stored, pending identification. The following tests were used for determination of the purified strains: Gram-staining, KOH test, production of oxidase and catalase (3% H_2O_2), and growth on appropriate media (Difco) (MacConkey agar, Baird-Parker agar, Pseudomonas agar, triple sugar iron agar). Final biochemical identification was carried out with API NE, API 20E, and API Staph (BioMerieux Vitek, France), according to Vanderzant and Splittstoesser *(7)*.

AMTF: Cultivation of Bacteria

Cultivation of bacteria formed part of a larger study of other wastes and cow manure; it used the thermophilic (55°C) anaerobic digestion procedure practiced in our laboratory *(1,8)*. We used an anaerobic methanogenic thermophilic bacterial population from our previous experiments on cow manure containing 10–12% solids and adapted it to fish and, separately, to poultry waste. The bacteria were cultivated under anaerobic conditions for 3 wk to be fed on fish or poultry waste at a constant temperature of 55°C. The digestion system for each waste comprised three anaerobic reactors, each being a 5-L plastic container fitted with a gas outlet and a feed inlet. The working volume was 4 L. The system was operated under batch feeding conditions: three kilograms of tuna or sardine waste or of deboned poultry waste (meat and bones) diluted to 15% solids was added to 1 L of the inoculum. Digestion (in three replicates) was established under thermophilic conditions (55°C) in a water bath and was operated continuously, with shaking for 5 min every hour. Biogas was collected and measured daily and its volume corrected to standard temperature and pressure (STP).

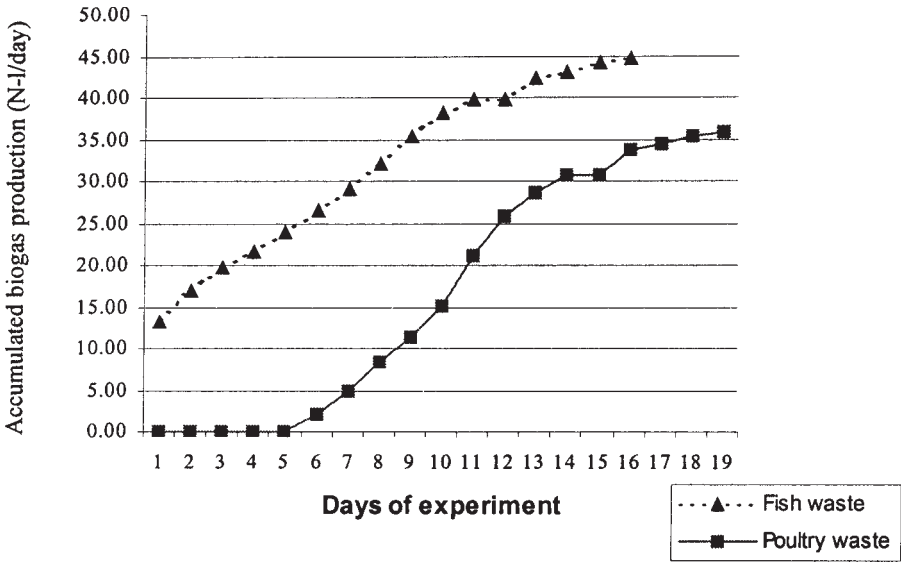

Fig. 2. Average accumulated biogas production (corrected to STP) from poultry and fish wastes in a batch AMTF system for organic wastes.

Results and Discussion

AMTF was applied to the wastes from local fish- and poultry-processing plants. The wastes as received contained 27–60% solids and were diluted with water to 15% solids. Plans are being made to use sewage from the plant in the future. The immediate aim was to establish the optimal conditions for stable fermentation by working with 15% solids and measuring the biogas production and the composition of the residues. In some cases, the anaerobic digestion was associated with bad odors, typically when fermentation conditions were poor. The biogas generated burned with a blue flame but had an odor, probably because of high contents of H_2S and mercaptans.

The procedure is still in the phase of establishing the best inoculum for the high-solids-content poultry wastes, and the results of the digestion are presented in Fig. 2.

Biogas production developed slowly, the yields were not high, and the degradation of this 15%-solid fish and poultry wastes was not sufficiently effective. One reason was the presence of bones and other indigestible materials in the waste, but we consider that the main reason was the very high protein (nitrogen) content. It is necessary to adapt the culture to this high nitrogen content or to change the C/N ratio by adding other wastes with high carbon contents (e.g., paper). Salminen et al. *(9)* showed that in batch fermentation there is an inhibition of degradation by propionate, a finding indicating that additional work is needed to render AMTF effective. Simply to mix in additional carbon sources by bringing other materials into the food-processing plants could cause problems for a food-processing complex.

Table 1
Bacterial Content of Various Animal-Processing Wastes Before and After AMTF from Biogas Degradation[a]

Type of waste	Total counts (CFU/g)	Composition of microbial flora
Poultry meat	$8.2 \times 10^5 \pm 7.0 \times 10^1$	*Staphylococcus aureus*; *Micrococcus* spp.; *Aeromonas hydrophila*; *Shewanella putrefaciens*; *Pseudomonas* spp.
Poultry-processing waste	$7.1 \times 10^8 \pm 1.4 \times 10^2$	*Micrococcus* spp.; *Proteus vulgaris*; *Pseudomonas* spp.
Poultry-processing waste after digestion	$7.0 \times 10^5 \pm 7.8 \times 10^1$	*Bacillus* spp.—pure culture
Tuna-processing waste	$5.5 \times 10^9 \pm 3.1 \times 10^2$	*Staphylococcus* spp.; *Micrococcus* spp.; *Escherichia coli*; *Aeromonas hydrophila*; *Pseudomonas* spp.
Tuna after digestion	$4.0 \times 10^5 \pm 1.8 \times 10^2$	*Bacillus* spp.—pure culture

[a]Data are the mean of four replicates of one determination.

The heterogeneity of the raw materials, caused by the variability in the composition of the animal-processing plant outputs, and the high protein content of its high-solids wastes, created problems for the fermentation operation, and there were variations of up to 100% in gas output when different materials were handled.

Since the hygienic aspects of using the digested slurry for breeding fly larvae was a key factor in our decision to evaluate the use of anaerobic digestion as a precursor step to growing the larvae on wastes, we examined the bacterial contents of the slurry before and after digestion. The results of the bacterial content measurements before and after AMTF are presented in Table 1.

It is quite clear from these results that the anaerobic thermophilic digestion eliminated all pathogenic bacteria examined. This finding is important for deciding whether or not to use methanogenic anaerobic thermophilic bacteria to digest the wastes prior to using them for rearing flesh fly larvae. The antibacterial activity of the larvae would probably intensify the elimination of the pathogenic bacteria.

The data on breeding housefly (*M. domestica*) and flesh fly (*P. sericata*) larvae (Table 2) are based on the raw materials before the anaerobic fermentation. The residue from the thermophilic process will be examined in the near future, to determine its suitability as a substrate for breeding larvae. Results of several experiments in which different numbers of eggs and different quantities of wastes were tested are presented in Table 2.

Table 2
Productivity, Expressed as Conversion of Wastes to Larvae, in Rearing Housefly and Flesh Fly Larvae on Animal Wastes[a]

	Yield (%)	
	Average	SD
Housefly larvae grown on rearing medium	10.00	NA[b]
Flesh fly larvae grown on poultry waste	22.47	3.89
Flesh fly larvae grown on fish waste	35.34	12.42

[a]Experiments were performed with various ratios of eggs and waste materials in order to define the optimum conditions and maximum conversion efficiency. Averages on the same egg-per-gram-of-waste basis are presented.
[b]NA, not available.

Table 3
Changes in Chemical Composition of Housefly Rearing Medium and Larvae Raised on It

	Wet matter (%)		Dry matter (%)			
Sample	Water	Dry matter	Proteins	Lipids	Carbohydrates	Ash
Rearing medium	44.6	55.4	19.9	11.6	61.4	7.2
Composition of larvae	76.7	23.3	57.5	23.7	11.2	7.7
Remaining substrate	31.4	68.6	27.7	10.5	50.0	11.8

It was found that the digestion by flesh larvae depended on the number of eggs per unit weight of substrate and varied considerably. When poultry waste was used, we obtained an average yield of 22.47% and the reduction in weight was up to 30–52%. When fish waste was used, we obtained a yield of up to 50% and the reduction in weight was up to 30–64%. In addition, bigger larvae were obtained from fish waste than those raised on poultry waste, and their development took 2 to 3 d less on fish waste.

The rearing medium and the fish waste, and the larvae grown on them, were chemically analyzed. The results are presented in Tables 3 and 4. Data are not available for the experiment done on the poultry waste.

It is clear that during the digestion by flesh fly larvae a high percentage of the waste was digested and that the larvae produced could be used as a resource for applications that are yet to be defined, but that could include chick or fish feed, or medicinal uses. The use of larvae for medicinal purposes is recognized to have beneficial effects on wounds and has become more accepted in recent years; debridement, or elimination of necrotic tissue, is one mechanism by which wounds can be cleaned. The high yields obtained from wastes, accompanied by the elimination of pathogenic bac-

Table 4
Changes in Chemical Composition
of Samples of Fish Waste Used as Substrate and of Flesh Fly Larvae Raised on It

Sample	Wet matter (%)		Dry matter (%)			
	Water	Dry matter	Proteins	Lipids	Carbohydrates	Ash
Fish waste as substrate	71.6	28.4	58.5	36.6	0.3	4.6
Composition of larvae	72.1	27.9	52.0	32.6	10.8	4.7
Remaining substrate	63.0	37.0	64.1	30.5	0.0	5.4

teria, could be of commercial importance. Although the wastes include both bones and meat, and the larvae can utilize only the meat, we have, nevertheless, found that rearing larvae provides a prolific source of proteins that could be important as a chick and fish feed. The findings of our research can serve as a basis for applying the results on a large scale. Beyond the present work are both an enormous challenge and an enormous opportunity. The challenge is to develop a sustainable economy that the planet will be capable of supporting indefinitely. This research presents an opportunity to develop a different and innovative way of thinking about wastes, enabling them to be regarded as a resource from which to produce new and profitable products, rather than as a problem to be disposed of.

Acknowledgments

We wish to express our gratitude to Y. Hanun for his help with the fermentation system; to A. Chizov-Ginzburg for assistance with the rearing and storage of the fly larvae; and to L. Glatman, V. Drabkin, K. Chechik, and I. Gabay for the bacterial and chemical analyses. We also wish to thank the Off-Hagalil poultry processing plant in Kiryat-Shmona and the Yona fish processing plant in Tirat-Hacarmel for the wastes used in the experiments.

References

1. Klinger, I. and Marchaim, U. (1987), *Isr. J. Vet. Med.* **43,** 181–187.
2. Zenkevitch, L. A. (1969), *Prosveshenie* (Moscow) **3,** 516–518 (in Russian).
3. Prete, P. E. (1997), *Life Sci.* **60,** 505–510.
4. Casu, R., Eisemann, C., Vuocola, T., and Tellam, R. (1996), *Int. Parasitol.* **6,** 623–628.
5. Wijffels, G., Gough, J., Muharsini, S., Donaldson, A., and Eisemann, C. (1997), *Insect Biochem. Mol. Biol.* **5,** 451–460.
6. Cunniff, P., ed. (1995), *Official Methods of Analysis*, 16th ed., Association of Official Analytical Chemists (AOAC), Gaithersburg, MD.
7. Vanderzant, C. and Splittstoesser, D. F. (1992), *Compendium of Methods for the Microbial Examination of Food*, American Public Health Association, Washington, DC.
8. Marchaim, U., Kostenberg, D., and Epstein, E. (1997), *Mikrobiologiya* **66,** 706–711.
9. Salminen, E., Rintala, J., Lokshina, L. Y., and Vavilin, V. A. (2000), *Water Sci. Technol.* **41,** 33–41.