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Chapter 14

MEAT INDUSTRY HYGIENE, OUTLINES OF SAFETY AND MATERIAL RECYCLING BY BIOTECHNOLOGICAL MEANS

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ABSTRACT

Human civilizations have increased both in terms of the population densities and regarding the consequences of the development of various technologies, particularly that of the information technology and the transportation. However, the societies are increasingly vulnerable and dependent on the effective food production for the millions. Then the mass production requires solutions in industrial scale, and consequently also the problems can emerge in a massive proportion, if things turn into disastrous or less desired directions. Then the recycling of wastes and side streams could provide solutions for the hygienization and reuse of the biomass substances.

Microbiological meat and slaughterhouse hygiene has to be developed into:

1. rapid alertness,
2. means for recognition, confinement and eradication of any emerging situation, and
3. responsible circulation of organic matter within the industry.

In this book, a few chapters have been directed towards finding the solution for the hygiene maintenance of the meat industries. It is of our goal for the common good to face these issues boldly in order to facilitate the sustainable future.

1. INTRODUCTION

Since the onset of the human race, our ancestors have been so called hunter-gatherers (Harari, 2014). Meat has been produced to fulfill the needs for metabolic building blocks and energy of the human body system – and vitamins and trace elements.

At the same time with the meat and meat products being recognized as parts of the traditional diet, a multitude of microbiological threats are also emerging (Hakalehto, 2015a). The zoonotic diseases can easily spread via the meat production facilities, if inadequate attention is paid to the hazards caused by them. Also, the requirements of the deli production set up entirely new challenges (Simmons et al., 2014; Wang and Shen, 2015; Wong et al., 2015). Such solutions as a fluorescent powder has been suggested for the hygiene-control of the Ready-to-eat (RET) foods (Sirsat et al., 2014). In case of *Listeria monocytogenes* in a meat model system, such preservatives as sodium nitrate, chloride, acetate and lactate, as well as calcium propionate were found to inhibit bacterial growth in ham (Dussault et al., 2016).

Serious threats also emerge in the form of hazardous variants such as *E. coli* 0157 (Gurman et al., 2015), or antibiotic resistant strains, as well as obscure or sensational strains – like cases, such as the clinical outcomes of the "flesh-eating bacteria" (Makthal et al., 2016). Many agonizing, hygienic nightmares of highly dangerous viral epidemics associate with transmission with different animals as vectors for several highly contagious diseases, such as rabies, influenza, Ebola, SARS, Hantavirus, Marburg virus, swine and bird flu or HIV (Blaser, 2014). These epidemics most likely did not occur during the times of our hunter forefathers who survived or died on the basis of the availability of enough or not enough catches in their everyday life. They also had developed means for maintaining the high levels of food hygiene and safety on the basis of practical experiences (Hakalehto, 2015b). The modern meat processing industries, whose business is controlled by legislation and directives, take mostly the steps that are required by the legislator for their activities. This includes also proper means for waste management, recycling and rendering units (Figure 1).

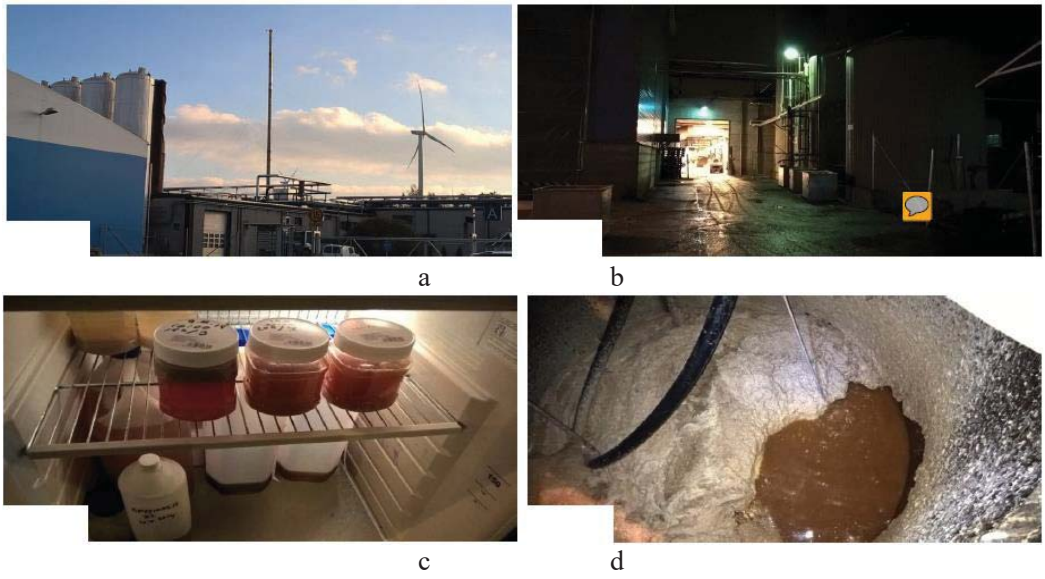

Figure 1. (Continued).

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Figure 1. a) Honkajoki Oy factory site in western Finland, where the rendering of meat-derived wastes take place. b) The waste treatment unit at the factory. c) Samples from Finnoflag Oy's experimental runs. d) View from inside the bioreactor during the process, as the solids accumulate to the surface. e) Test equipment, 5 m^3 bioreactor in the background. f) Storage area for the side stream fractions.

In fact, it is an urgent matter of the entire society to handle microbiological problems in a proper way. In a demonstrative experiment conducted by Dr. Lars Engstrand in Sweden, a common skin bacterium, *Staphylococcus epidermis*, and a common intestinal bacterium, *Enterococcus faecalis* produced an increase of resistance against antibodies during the eradication of *Helicobacter pylori* by antibiotic clarithromycin (Blaser, 2014). These bacteria happen to be highly prevalent strains in the meat production. Therefore, it was an alarming result that the number of the resistant strains did increase, not only acutely, but for the follow up period of 3-4 years. These bacteria are not necessarily dangerous pathogens for a healthy individual but mostly members of the commensal microflora. However, in case of immunocompromised, diseased or otherwise weakened patients, they could form considerable fatalities (Pesola and Hakalehto, 2015). This is particularly relevant, if the antibiotic

resistance will spread among the microbiomes. A similar kind of case of multiresistant strain was isolated from the hospital equipment in a pediatric ward (Hakalehto, 2011). The ciprofloxacin-resistant *E. coli* strains were isolated from a bathing water from a popular beach near Stockholm in Kallhäll.

In these chapters we wish to provoke ideas, how the waste treatment in the meat industries, as well as in the hygiene control should be arranged in order to properly deal with the issues of hygienic safety.

In fact, as the picture on the residues from the meat production turn into plant nutrition in one and same factory, this demonstrates the means and methods for the implementation of the circulation economy, as well as the integration of the industries with the agricultural production.

All the advancements in the field of controlling the nutrient flows and the microbes in them will attain more consciousness on the potentials for establishing sustainable industries. Also the direct positive contribution of this progress to human health and nutrition should become measurable.

2. Development of Microbiological Industrial Tradition

All areas of industrial progress need increasing understanding on the multidimensional character of scientific and technological development. Vast amount of experience has to accumulate on the manufacturing principles and the practices. This is particularly true in the case of the microbiological bioprocesses.

In a situation where microbial cell or cells live in a liquid broth or suspension, there are numerous potential biochemical reactions to be produced by the cell's metabolic machinery. This is the case, if the cell has initiated active metabolic mode (see chapter 21). However, in biotechnology, it is the purpose to generate a flux-generating reaction (Newsholme and Leech, 2011). The desired direction of this "reactional flow" is determined by the goals that strive the industrial unit in question.

Because it is often hard to maintain the operational integrity of a microbial culture or strain or community, or any other biocatalyst, this somewhat vague grounds for the biological production processes causes extra challenges when it is compared with the traditional chemical industries. However, in some sense these aspects of developing the bioprocesses can also give extra potentials provided that they are prudently managed.

The versatility of the reaction or reactivity in a bioprocess make up continuously emerging new options for research and development. The complexity of production matrices poses many operational challenges, in turn. It can be postulated that even up to 100% similar conditions in any complex microbiological system gives clearly variable results from time to time.

This new industrial revolution based on such ideas as artificial intelligence, continuous innovation, scientific intuition, liberal testing of ideas, etc. could at best become the mainstream of industrial development in the transition to the circulation economics. See also chapter 17 of this book.

The direction of a biological reaction can be measured on the basis of the gas emission from the production broth (Hakalehto, 2015c). The principles of ABOWE project (EU Baltic

Sea Region 2012-2014, www.abowe.eu) have been implemented into the carboxylate production in Poland (den Boer et al., 2016) and for converting amino acids and lipids into biochemical from the slaughterhouse waste in Sweden (Schwede et al., 2016).

3. INTRODUCTION TO THE BIOREFINERY ACTIVITIES

The microbiological industrial hygiene deals with the cleanliness of the processes, personnel and equipment. Main objectives of industrial hygiene maintenance are the acceptable microbiological quality on one hand, and the avoidance of harmful environmental emissions, or microbial loads, on the other. Two major lines of hygiene monitoring and safeguarding can be identified, namely:

1. adequate microbiological purity and safety regarding the products, personnel and the customers, and
2. keeping up correct microbial strains, cultures, communities and activities in the processes, where the microbes are utilized for the product formation, or modulation.

In the industrial microbiology, the scope of inspection or evaluation should not be the presence or absence of microbes in general or specific types of the micro-organisms in particular. Instead, different microbiological balances should be characterized, analyzed and followed up.

In the natural ecosystems microbes perform important functions in recycling matter. Their enzymatic and metabolic capabilities are formidable in degrading macromolecules. These capabilities should be better understood and exploited for achieving zero waste situations, as well as ecologically sustainable economics. Such efforts are underway and form a global trend also for restricting the climatological consequences of various industries.

One example of a successful implementation of microbial biotechnology is the production of 2,3-butanediol, which is an important platform chemical and useful raw material for synthetic rubber, plastic monomers, fiber, textiles, anti-icing substances and many cosmetics (Hakalehto et al., 2013a). Its production is carried out in South China using the technology of LanzaTech Ltd. from New Zealand (Köpke et al., 2014). There the exhaust gases of iron works, particularly the carbon monoxide, are directed into the bioprocess fluid where the carbon is assimilated from these emissions. By such approaches, it is possible to build up circulating bioeconomics within the industries.

This kind of development has been the goal in the six nation European Union Baltic Sea region biorefinery project, ABOWE (Implementing Advanced Biological Concepts for the Utilization of Waste). This project comprised of participants from Germany, Poland, Estonia, Lithuania, Sweden and Finland, and the author served as a key technology provider in bioprocess solutions and microbiological applications (see also www.abowe.eu). The results from the ABOWE experimentation are presented also in the chapters 10, 11, 13, 15 and 20 of this book.

As a tool for the novel biorefinery experimentation, a biorefinery Pilot A was designed and constructed in Kuopio, Finland, according to the ideas from several biorefinery projects by Finnoflag Oy with respect to combining the microbiological and enzymatic know how in

the process technology with hardware and software. The ABOWE project gave a proof of concept on the utilization of three different waste mixtures in three countries, namely Finland, Poland and Sweden (see also the chapter 11). The field trials at the testing sites took place two months each.

Independently of the ABOWE experimentation which aimed at demonstrating the functionality and usability of biorefinery ideas for sustainable waste utilization in different industries, the biorefinery activities of Finnoflag Oy have consisted of efforts in various industries (Hakalehto et al., 2013a, b; del Amo and Hakalehto, 2015; Hakalehto, 2015a). The main ideas behind the process design have related to giving a proof of economic feasibility combined with ecological sustainability by using microbial communities or strains which circulate the matter in Nature. As a result, no wastes are produced but these side streams can become valuable raw materials and assets. One example of such natural sources of valuable substances or activities is the production of mannitol by rumen contents collected in a slaughterhouse (Hakalehto, 2014; del Amo and Hakalehto, 2015).

4. Lactate as a Substrate in the Industry and in the Food Products with Functional Links Also to the Neuromicrobiology in the Human Body

Lactic acid (lactate) is the major natural product of many so called lactic acid bacteria. They are Gram positive facultatively anaerobic cocci or rods. The members of the group often colonize the intestines of humans or warm-blooded animals, as well as the surfaces of many plants. They are rather demanding in their nutritional requirements (Rodríguez et al., 2010), and tend to grow better in relatively anoxic environments. This may be due to their rather slow and metabolically challenging mode of growth, which makes them vulnerable and subjected to competition by more robust members of the flora. However, where milk is present, they often get a competitive edge, and many strains have been used traditionally for fermented food and drinks. These bacteria remain in high numbers in the slaughterhouses and the food production industries (Barrangou et al., 2002). They also colonize human intestines, skin and some other tissues (Albesharat et al., 2011).

Also in the rumen, the lactic acid bacteria constitute one of the most important groups. The homofermentative strains produce only lactate from lactose, whereas the heterofermentative ones make several end-products (Barrangou et al., 2002). In the rumen environment it is likely that the lactate produced is converted into propionate and other organic acids (Figure 2) (Hakalehto, 2015d; Hakalehto et al., 2013a). In a medium with some particulates, the rod-shaped propionic acid producing bacteria with obligately anaerobic cells neither do not attach to any solid structures nor form aggregates (Hakalehto, 2015e). This indicates their mode of metabolism which utilizes the dissolved lactate instead of attacking on any macromolecular structures. In the intestines, the propionibacteria are located in the furthermost end of the intestines in the areas of descending colon and sigmoid gut as well as the rectal areas (Hakalehto, 2015f). In these parts of the alimentary tract, the anaerobiosis is perhaps the most complete one.

Quite recently some discussion has been going or with respect to the general health in relation to the body's biochemical and microbiological "resources." This could give a hint on

the mechanisms of the various neurolipid products regenerating the brain functions. Such extracts as NeuroWay™ (Biomed Oy, Helsinki, Finland), have shown to protect and reform the lipid membranes. So it could perhaps be justified to establish and strengthen a new discipline in the microbiological studies, namely the field of neuromicrobiology. It should also be taken into account that propionate is known for its tendency to dissociate protein fibrils, such as fimbria (Rhen et al., 1983). This effect of propionic acid on the protein fibrils, combined with the high affinity of *Propionobacterium* sp. toward glycerol (a key substance in lipid membranes) could lead to undesired destruction of nerves and neural connections. In slaughterhouse practices, these effects of the propionic acid could explain its usefulness as a preservative.

If applied in a correct way and with adequate caution the neurolipids could provide a tool for protecting and reviving the damaged neural, lymphoid and other tissues. In studies with flagellar N-terminal peptide structures resembling in electron micrographs closely the β-amyloid peptide of Alzheimer's disease and various other prions e.g., in the Creutzfeld-Jacobi disease (Hakalehto et al., 1997). When a hybrid peptide originating from a linkage between the N-terminal flagellar sequence of *Pectinatus sp*. with some fimbrial peptide sequences of *Salmonella* was produced, it caused epileptic episodes in rabbits and hairless lesions and tumors in rats (Hakalehto, 2015e). With respect to the meat industries, it could be possible to produce health related products from their side streams, such as lipid products. However, utmost hygienic caution and care has to be exercised when such products are launched.

Debris from the degraded proteins and lipids could cause imbalances in the normal microflora, and it can possibly give room to some in-house or intruding opportunistic pathogens. A commensal *Staphylococcus haemolyticus*, for example, was isolated as an intruding strain in the peritoneum of a severely sick young patient where it developed lipid formation around the bacterial aggregates of exceptionally small cells (Hakalehto, 2015b). This kind of overgrowth and microbiological dysfunction could lead to chronic diseases and pain (Kokki, 2015). It has been postulated, that such common microbes as *Staphylococcus aureus* may directly activate or inhibit the human cell receptors (Kang et al., 2015; Posner et al., 2015). This provokes a question about the relations and potential role of the food or the environment in worsening inflammations and painful chronic conditions. Therefore, it is not meaningless, what strains are disseminated by the industries into their products or into environment. Microbial strains that may look out harmless ones could also contribute to various disorders, tissue malfunction, immunological disturbances, etc. All these adverse effects and threats caused by imbalanced normal flora were highlighted in the lecture given by the author in Stockholm, during the Swedish Laboratory Days in 2012, a seminar with audience from all over Sweden, arranged by Tom Pettersson, PhD, who is a former head of R and D of Capio Diagnostics Ab.

Since the direct consequences of negligence in the industrial hygiene control can be seen as threats to public health, it is necessary to develop counter-measures against the infections or harmful agents, such as the antibiotic resistant bacterial strains belonging to the family *Enterobacteriaceae*. For example, a harmless species of *Enterobacter cloaceae* had developed a multi-resistant form, which was distributed at a pediatric ward by the contaminated equipment (Hakalehto, 2011). Such strains could potentially transfer the resistance genes to other bacteria.

The general hazards of the versatile pathogenic organisms becoming resistant to antibiotics are recognized as one of the major threats to our civilization (Mackenzie, 2013). To confine and eradicate the problem we would need:

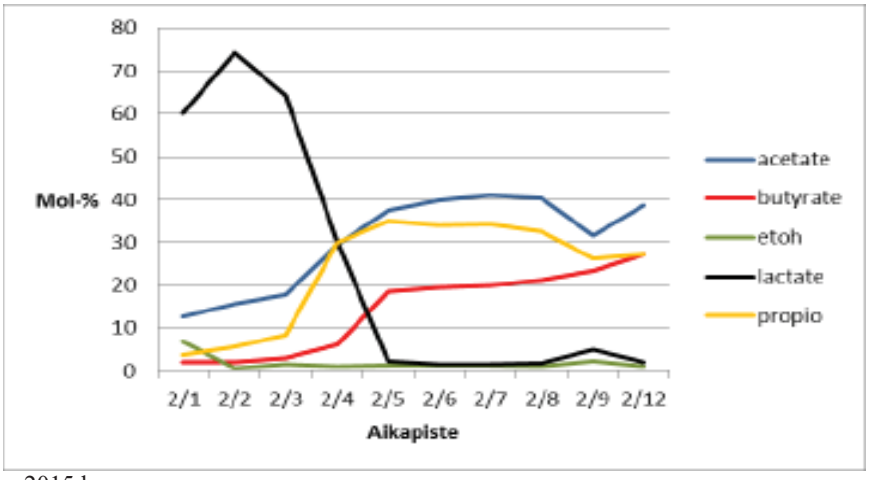
- new antibiotics
- limitations (sensible ones) in the use of antibiotics in medicine, agriculture and industries (in order to avoid the formation of antibiotic resistant strains or epidemics)
- better hygiene control
- new tools for the analyses
- information on the epidemics
- public awareness

For example, carbapenem-resistant *Enterobacteriaceae* (CRE), disease-causing multiresistant gut bacteria, are spreading all over the globe (Hawkey, 2015). The worst killer among the contagious diseases, tuberculosis, is also spreading as multiresistant strains (Ekaza et al., 2013). Proper diagnostics and counter-measures are warranted. We have developed enhanced mycobacterial detection by using the PMEU (Portable Microbe Enrichment Unit) (Hakalehto, 2013).

5. The Production of Propionic Acid by *Propionibacterium* Sp

The optimal growth conditions in a chemostat for *Propionibacterium acidipropionici* (Quesada-Chanto et al., 1994):

– temperature at +37°C
– pH 6.5
– complete anaerobiosis



Hakalehto, 2015d.

Figure 2. The conversion of lactate into propionic acid is mainly facilitated by *P. acidipropionici* in slaughterhouse waste. Conversion to other organic acids and ethanol is expressed as molar percentage and it was also achieved by mixed flora in the wastes.

Between the sucrose range within 30-170 g/l in the fermentation medium, no significant substrate inhibition occurred. For the combined production of propionic acid and vitamin B12, of 1.5 mg $FeSO_4$-7 H_2O per gram of dry biomass, 0.75 mg of cobalt ions, 0.3 mg benzimidazole, and 12 mg of yeast extract, each added to the same dry biomass, turned out to be necessary additions to the sources of nitrogen, phosphate and magnesium ions. Moreover, an extra addition of up to 2.8 g of betaine into the gram of dry biomass significantly increased the production of vitamin B12.

During the optimization studies described here, it turned out that the optimal conditions for the propionic acid production are different from the optimal conditions for the production of vitamin B12. In any case, this vitamin could constitute a valuable health associated product prepared from the slaughterhouse side streams. Quesada-Chanto and coworkers (1994) concluded that the optimal vitamin B12 production required:

- temperature at 40°C
- pH 6.5
- aerobic conditions (0.5 ppm aeration of 100 rpm)

There has been an interest in many occasions to produce propionic acid by bacteria from such low cost substrates as whey or molasses (Vivian and Yang, 1992). Its calcium, potassium and sodium salts are used as food preservatives. We managed to produce propionate into the 20% concentration of the dry weight of the fermentation broth (Hakalehto, 2015d). In this context it should be emphasized that *Propionibacterium acidipropionici* is considered as a safe production organism by EFSA (European Food Safety Authority).

6. Production of a Sugar Alcohol, Mannitol, by Using the Rumen Microbiota

Mannitol is a highly potential carrier material in tablets and medications, sweets, chewing gums, etc. (del Amo and Hakalehto, 2015). The microbiological production of mannitol has been attempted many times by pure cultures of various micro-organisms (Wisselink et al., 2002). However, it turned out to be more rewarding to produce mannitol by the entire community of the rumen microbes (Hakalehto, 2014).

6.1. Production of Mannitol Using Ruminal Microbia

One significant fraction of abattoir waste is so called rumen digesta which means the partially undigested content from a rumen of an animal to be slaughtered. One slaughtered cow provides at least 50 kilos of rumen digesta. What is essential is that this material contains abundant amounts of various natural microbes which participate in the nutrition intake in the cow digestion. These microbes create naturally versatile cultures. The material in question is a valuable, biotechnical raw material. Each day tens of tons of it is produced by a large cow abbatoir. The rumen content is not classified as manure. It is a potential organic raw material.

The aim of the project carried out in several industrial settings was to initiate pilot-scale experiments to produce mannitol using the microbes from rumen. Mannitol is a nontoxic, sugar alcohol which can be used for several purposes i.e., as an important component in candies, chewing gums, pastilles and pastries. It has a low calorie amount which makes it an attractive alternative for sugars. Moreover, mannitol has potential uses in the field of medical industry, for example, quickly dissolving oral pills are planned to be increasingly produced in the future with this material in medicines because of e.g., its quick dissolution in mouth (Rajpurohit et al., 2011; Labib, 2015). Formulation containing gelatin-mannitol 3.75% (w/v) and 3.5% (w/v), respectively disintegrated in less than 10 seconds (Gugulothu et al., 2015).

Mannitol gives a pleasant mouth feeling for various orally taken medicines (Jacob et al., 2009). Such big pharmaceutical companies as Merck KGaA (Darmstadt, Germany) have researched the use of mannitol as an excipient in solid dosage forms (Ohrem et al., 2014).

At the moment the price for rectified mannitol varies up to between 15 and 75 dollars per kilogram for medical use. Raw mannitol material as a substrate for food industry costs a few euros per kilo. It is commonly used as a major constituent of chewing gums and pastilles and as a carrier material in medicines.

After the mannitol process the rumen contents can be further used as raw material for other biotechnical processes. Lactate, propionate and other organic acids or alcohols can be collected from it using a tested method. The residues of the mannitol process are suitable for the production of organic fertilizers and hygienized and safe biomasses can be combined into the substrates.

6.2. Procedures and Results

6.2.1. Laboratory Scale Experiment in 10 Litre Containers

Fresh nutrition under digestion taken from a rumen of a just slaughtered cow and molasses mixed with water in proportion of 1/10 (Farmer's mixtured molasses, Suomen Rehu Oy) were used main source materials.

The experiment was carried out in two containers so that no other microbes except those coming from the rumen digesta were added to one container (Container I) and to the other one *Klebsiella mobilis* bacteria were added (Container II).

The containers were held in a temperature of +37°C and sterilised room air was pumped into their bottoms with a perforated tube. Samples were collected from the containers every 2 to 5 hours. At intervals during the experiment, molasses and cane sugar were added.

Cultures were made from the 41 hour samples onto CHROMagar™ Orientation™ dishes (Becton, Dickinson and Co., US). The results of the culturing are displayed in the Table 1.

Figure 3 displays the mannitol concentrations (mg/ml) in container I in the beginning of the experiment and after 20 and 41 hours. The turquoise colonies are potential *Klebsiella*-bacteria.

Figure 4 displays the concentration of mannitol (mg/ml) in the container II in the beginning of the experiment and after 20 and 41 hours.

Amounts of mannitol, different organic chemicals and the remaining sugar raw material were measured using NMR-method and GC-MS (gas chromatograph with mass spectrometer) for 2,3-butanediol.

Figure 5 illustrates the accumulation of 2,3-butanediol based on GCMS measurement method. This is an example of one possible side product fraction that can be made as a side product of mannitol.

Table 1. Colonies growing during the microbiological testing on the CHROMagar™ Orientation- dishes after an incubation period of two days (+37°C)

	dilution -6	dilution -7	dilution -8	
Container I	full of light reddish and turquoise growth	41 dim, very small, reddish and 25 dim and very small turquoise colonies	one light red colony	Resemble *Lactobacillus*-colonies
Container II	full of reddish dim and turquoise growth	30 dim, very small, turquoise/reddish colonies and 19 white, rounded ones	some very small, dim, turquoise/reddish colonies, 6 white rounded ones	

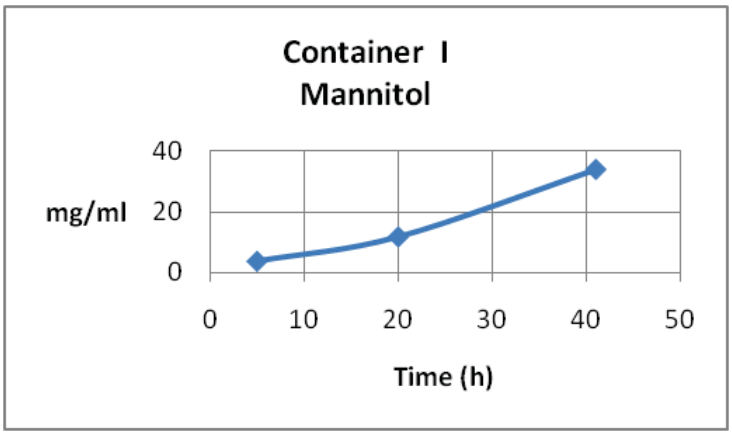


Figure 3. The concentrations of mannitol in the container I.

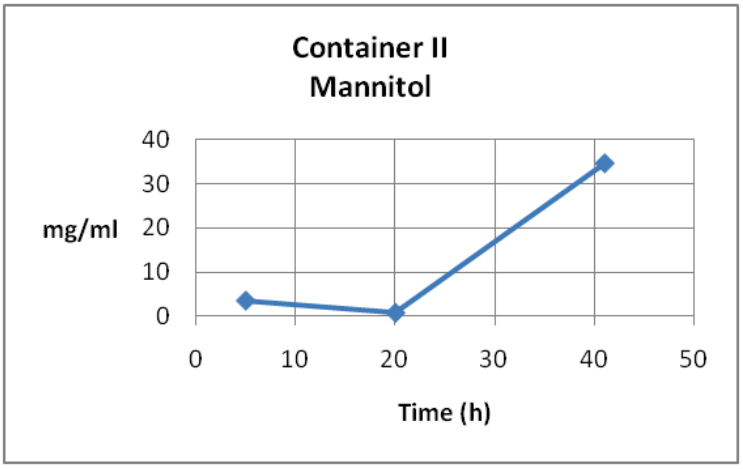


Figure 4. The concentrations of mannitol in the container II.

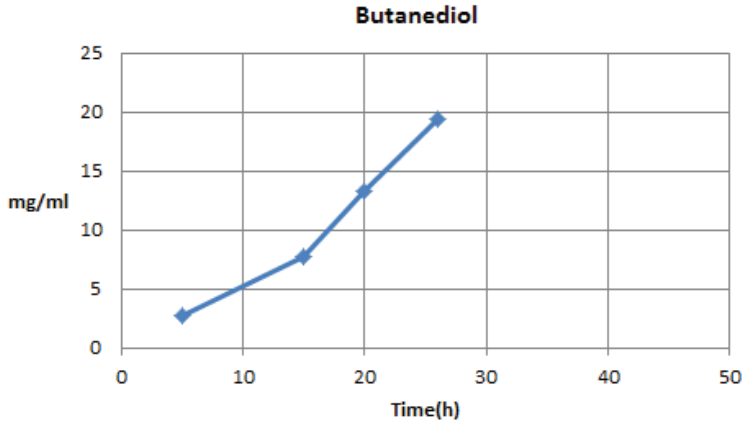


Figure 5. The concentration of butanediol in the bucket without *Klebsiella* inoculum.

Photos: Toni Hirvonen.

Figure 6. The reactor tank (on the left) and its empty interior (on the right). The process run was performed using fresh rumen digesta collected from the slaughtered animals in a controlled fashion. Potential products were hygienized after their collection.

6.2.2. The Pilot-Scale Experiment

Tests were carried out in a reactor tank of 5 m^3 (Figure 6). The tank was heated with hot water which circulates in a plastic tube inside the tank. Compressed air/gas from the pressure bottles is lead to the bottom of the tank with a perforated tube.

At first 600 litres of water and 60 litres of straw pellets were taken into the reactor. The pH value of the mass was around 7 and it was adjusted with formic acid to 5,1. The temperature was raised up and at +54°C 0,8 litres of Optimash™ enzyme solution was added. The hydrolysis time was 3,5 h. After that 1 m^3 of fresh rumen digesta (Figure 7) was mixed with water at the temperature of +40°C. The mixture was sparged with a small amount of air, and the pH adjusted with granular garden lime.

Another 500 kg of molasses were added into the reactor, together with some adjustment. After 25 hours the pH had lowered to 4,6. It started to increase by adding sodium carbonate dissolved to water. A total of 17 kg sodium carbonate was added during the experiment. After

the additions the pH value of the mass increased to 5,9. After adjusting the pH, a part of the mass rose to the surface and created a thick, hard lid which covered the liquid phase and was containing only minor amounts of solid materials. The experiment lasted 48h. Samples were taken in every 2 to 4 hours.

Figure 8 displays the concentrations of mannitol during the experiment.

6.2.3. The Pilot-Scale Experiment in Honkajoki

The mannitol process was performed with patented processes and protected with some technological know how about the microbiological set up (by E. H.). At first 500 l of hot water and 60 l of straw pellets were added into the reactor. The pH value of the mixture was 8.27. With the help of formic acid the pH was lowered to 6,14 and 1 l of Optimash™ enzyme was added. After 3 hours 2,1 m3 of fresh rumen digesta and 2 m^3 of water were taken into the reactor. pH was adjusted with grainy lime. The temperature of the reactor was kept between 36-38°C by using circulating hot water. After the rumen digesta had been in the reactor for 10 hours 500 kg of molasses with some supplement materials were added and after 15 hours 325 kg more of molasses was added. The pH value was raised after 30 hours and again after 44 hours. In connection with the latter elevation of the pH an additional 250 kg of molasses was added. Figure 9 displays the mannitol concentrations during the experiment. The aim of this second pilot-scale test was to maximise the production of mannitol.

6.3. Discussion and Conclusions

The best result from the mannitol process was 86,7124 mg/ml which corresponds 8,6% (w/v). The alfa- and beta-glucose levels were still a bit elevated which indicates significant remaining glucose levels in the broth. The highest concentration was at the second last measurement point (n:o 13). The level of about 8.5% was reached in the samples 10-13. This level was reached only after 5 h from the latest addition of molasses (44h - >49h). This is a relatively short time period of process time and a positive matter from the point of view of building a continuously operable process.

Photo: Anneli Heitto.

Figure 7. Addition of the rumen content.

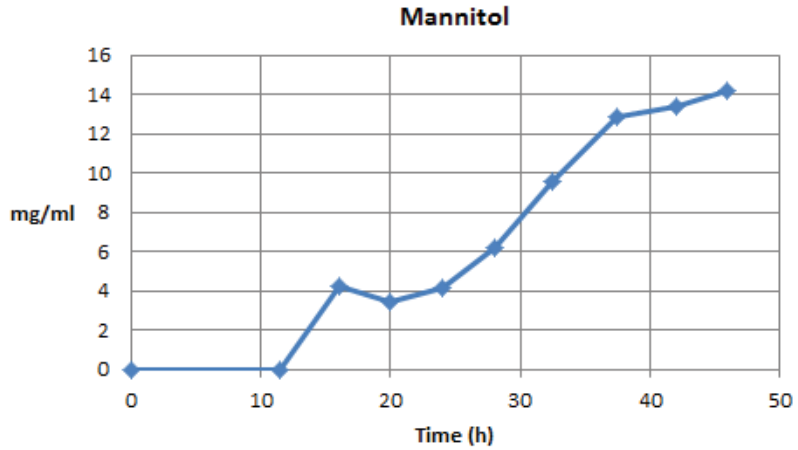


Figure 8. The mannitol concentrations during bioreactor experiment 1. The use of formic acid in adjusting the pH might have slowed down the onset of the production due to complicated microbiological adjustment factors.

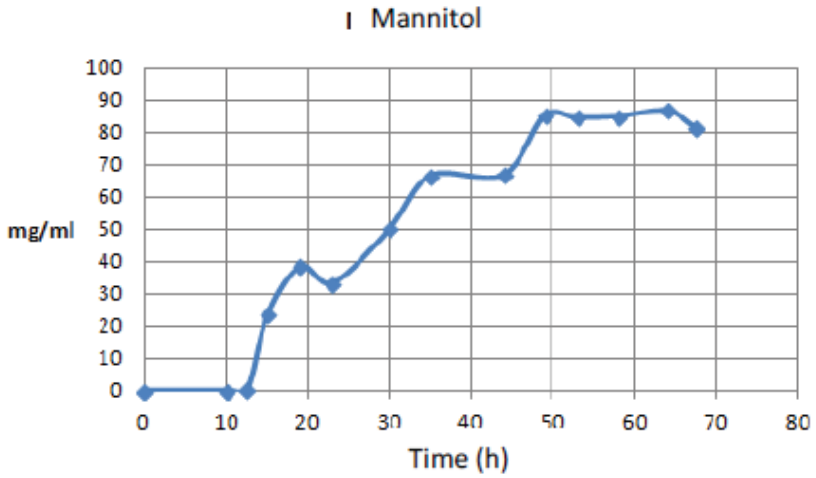


Figure 9. The mannitol concentrations during the bioreactor experiment 2. The outcome is based on the NMR results. The fact that the increase of mannitol stopped at about 8.5% (w/v) (85 mg/ml) probably indicates the shortage of some necessary trace elements from the reaction mixture because there were enough sugars left for the microbes to continue the process. Another possibility is that the osmotic pressure of the reaction solution began to limit the production. To avoid this it might be justifiable to dilute the solution during the final stage of the reaction.

Also methanol (MeOH) was formed, which was surprisingly high being about 1.5%. As a product it is not very valuable but it discloses the appearance of interesting bacterial strains in the population which could be utilized in the future for various processes. On the other hand, MeOH can be an inhibitor from the point of view of certain follow-up processes, for example the oxidation of nitrite into nitrate.

Based on the results above the calculated production is equal to about 258 kilos of the final product which, when purified to food quality is about 4 €/kg i.e., 1000€ for this three cubic metres batch. As a raw material for the pharmaceutical industry the price could be clearly higher after careful purification. Of this reactor content just over one third was rumen

digesta. With the current retail store prices the raw material expenses for molasses are 770 euros, in regards to the rumen digesta the gate fee could at least for the time being be calculated on the plus side. As a source for sucrose it is possible, from industrial sources, to get considerably cheaper raw material than the agricultural molasses.

7. Biorefinery Planning and Substantial Circulating in Biomass Processing/Their Contribution to Atmospheric Indicators

Remarkable atmospheric environmental contributors in the exhaust fumes of the oxides of carbon and nitrogen can be assimilated by the microbes in the biorefinery units. Also methane, which otherwise would get liberated into the air, is diminished as the microbial communities carry out the degradative reactions of various waste substances and biomasses. Following up the gas flows into and out of the bioreactor of the Pilot A experimental station constructed and tested in the six nation European Union ABOWE (Implementing Advanced Biological Concepts in the Utilization of Waste) Baltic Sea Region biorefinery project 2012-2014 could produce useful information about the climatological effects of the microbes in general and in the inner cycles of the mass and food industries, or agricultural circulation events. For further information, see the chapters 10, 11, 13, 15 and 20.

8. Strategies in Microbiological Utilization of Meat Waste

Since the animal waste and corresponding industrial wastes constitute a potentially hazardous material whose irresponsible management may lead to dissemination of zoonotic diseases, environmental threats, occupational hazards, etc., it is crucial that these residues or side streams are safely handled. This is the responsibility of the meat processing industries, and extensive legistation has been stipulated in all countries regarding this matter. Therefore, modern rendering units have been established in many countries.

Moreover, waste hygienization can lead to the formation of significant novel raw material sources and fractions, whose conversion to valuable energy compounds and biochemicals increases the potential value of these biomasses. This was one of the aims during the testing of the ABOWE pilot plant (Pilot A). See also the above-mentioned chapters. The design of this 12 m long movable unit is described also in chapter 10. In case of the treatment of the Hagby farm and ecological chicken abattoir waste fraction in Sweden it was proven out that the proteins in the wastes were converted into useful products, such as valeric acid (Schwede et al., 2016). This substance was also produced from the sorted biowaste during the Polish experimentation in Southern Silesia, in 2014. In case of the Polish tests, side stream carbohydrates were the main substrate. For the corresponding test results, see the chapter 13. In earlier testing with some Finnish cow slaughterhouse wastes, the formation of propionic acid from lactate was documented as mentioned above (see the paragraphs 5 and 6). Since the latter is a general product from the fermentation of various animal wastes, it was interesting to

observe this phenomenon in an industrial scale. This finding was previously reported also in the book, "Microbial Food Hygiene" (Hakalehto, 2015a).

9. RENDERING THE SLAUGHTERHOUSE WASTES

Mankind needs proteins for the nutrition. A big portion of them is derived from the plant kingdom. However, meat is important source of proteins. Such nutritional factors as vitamins or amino acids, are often obtained most readily from animal husbandry (Hakalehto, 2015g). Fisheries are also important sources of these nutritives and substances. The use of animal food sources brings along some hygienic problems (Armon, 2015; Hakalehto, 2015b). Avoiding them is one of the main tasks of the microbiological industrial hygiene. For example, such threat as botulism caused by the toxins of *Clostridium botulinum* could occur both in meat and fish processing industries and in their products (Pesola et al., 2015).

An important part of rendering industries is the elimination of hygienic problems caused by the protein-rich raw materials. Biorefining is often the proper treatment for the wastes, side streams and unused parts of the material flows of these industries. This usually involves the hygienization of the waste materials, as well as the reuse of these streams, which usually are not handled in the cold chain anymore. Also the dead domestic animals belong to the objects, which are to be treated in such a way that prevents any microbiological hazardous or harmful agents from spreading into environment, food production or population. In Finland, the legislator has given this task to the company, Honkajoki Oy, jointly owned by the two meat processing companies, Atria Oyj and HK Scan Oyj. In practically all countries, the renderization units of such kind deal with the carcasses and their parts.

Different wastes of animal origin are divided into three categories (European Commission Decree N:o 1069/2009, see Table 2).

Table 2. Categories of the animal by-products according to the European Commission Decree No: 1069/2009

I. Category 1 material comprises the following animal by-products (Article 8):
a. entire bodies and all body parts, including hides and skins, of the following animals: i. animals suspected of being infected by a transmissible spongiform encephalopathy (TSE) in accordance with Regulation (EC) No: 999/2001 or in which the presence of a TSE has been officially confirmed; ii. animals killed in the context of TSE eradication measures; iii. animals other than farmed and wild animals, including in particular pet animals, zoo animals and circus animals; 9002.11.41 NE Official Journal of the European Union 14.11.2009; iv. animals used for experiments as defined by Article 2(d) of Directive 86/609/EEC without prejudice to Article 3(2) of Regulation (EC) No 1831/2003; v. wild animals, when suspected of being infected with diseases communicable to humans or animals;

b. the following material:
i. specified risk material;
ii. entire bodies or parts of dead animals containing specified risk material at the time of disposal;
c. animal by-products derived from animals which have been submitted to illegal treatment as defined in Article 1(2)(d) of Directive 96/22/EC or Article 2(b) of Directive 96/23/EC;
d. animal by-products containing residues of other substances and environmental contaminants listed in Group B(3) of Annex I to Directive 96/23/EC, if such residues exceed the permitted level laid down by Community legislation or, in the absence thereof, by national legislation;
e. animal by-products collected during the treatment of waste water required by implementing rules adopted under point (c) of the first paragraph of Article 27:
i. from establishments or plants processing Category 1 material; or
from other establishments or plants where specified risk material is being removed;
ii. catering waste from means of transport operating internationally;
g. mixtures of Category 1 material with either Category 2 material or Category 3 material or both.

II. Category 2 material shall comprise the following animal by-products (Article 9):

a. manure, non-mineralised guano and digestive tract content;
b. animal by-products collected during the treatment of waste water required by implementing rules adopted under point (c) of the first paragraph of Article 27:
i. from establishments or plants processing Category 2 material; or
ii. from slaughterhouses other than those covered by Article 8(e);
c. animal by-products containing residues of authorised substances or contaminants exceeding the permitted levels as referred to in Article 15(3) of Directive 96/23/EC;
d. products of animal origin which have been declared unfit for human consumption due to the presence of foreign bodies in those products;
e. products of animal origin, other than Category 1 material, that are:
i. imported or introduced from a third country and fail to comply with Community veterinary legislation for their import or introduction into the Community except where Community legislation allows their import or introduction subject to specific restrictions or their return to the third country; or
ii. dispatched to another Member State and fail to comply with requirements laid down or authorised by Community legislation except where they are returned with the authorisation of the competent authority of the Member State of origin;
f. animals and parts of animals, other than those referred to in Article 8 or Article 10,
i. that died other than by being slaughtered or killed for human consumption, including animals killed for disease control purposes;
ii. foetuses;
iii. oocytes, embryos and semen which are not destined for breeding purposes; and
iv. dead-in-shell poultry;
g. mixtures of Category 2 material with Category 3 material;
h. animal by-products other than Category 1 material or Category 3 material.

III. Category 3 material shall comprise the following animal by-products (Article 10):

a. carcases and parts of animals slaughtered or, in the case of game, bodies or parts of animals killed, and which are fit for human consumption in accordance with Community legislation, but are not intended for human consumption for commercial reasons; 41/003L NE Official Journal of the European Union L 300/15.

Table 2. (Continued)

b. carcases and the following parts originating either from animals that have been slaughtered in a slaughterhouse and were considered fit for slaughter for human consumption following an ante-mortem inspection or bodies and the following parts of animals from game killed for human consumption in accordance with Community legislation:

i. carcases or bodies and parts of animals which are rejected as unfit for human consumption in accordance with Community legislation, but which did not show any signs of disease communicable to humans or animals;

ii. heads of poultry;

iii. hides and skins, including trimmings and splitting thereof, horns and feet, including the phalanges and the carpus and metacarpus bones, tarsus and metatarsus bones, of: — animals, other than ruminants requiring TSE testing, and — ruminants which have been tested with a negative result in accordance with Article 6(1) of Regulation (EC) No 999/2001;

iv. pig bristles;

v. feathers;

c. animal by-products from poultry and lagomorphs slaughtered on the farm as referred to in Article 1(3)(d) of Regulation (EC) No 853/2004, which did not show any signs of disease communicable to humans or animals;

d. blood of animals which did not show any signs of disease communicable through blood to humans or animals obtained from the following animals that have been slaughtered in a slaughterhouse after having been considered fit for slaughter for human consumption following an ante-mortem inspection in accordance with Community legislation:

i. animals other than ruminants requiring TSE testing; and

ii. ruminants which have been tested with a negative result in accordance with Article 6(1) of Regulation (EC) No 999/2001;

e. animal by-products arising from the production of products intended for human consumption, including degreased bones, greaves and centrifuge or separator sludge from milk processing;

f. products of animal origin, or foodstuffs containing products of animal origin, which are no longer intended for human consumption for commercial reasons or due to problems of manufacturing or packaging defects or other defects from which no risk to public or animal health arise;

g. petfood and feedingstuffs of animal origin, or feedingstuffs containing animal by-products or derived products, which are no longer intended for feeding for commercial reasons or due to problems of manufacturing or packaging defects or other defects from which no risk to public or animal health arises;

h. blood, placenta, wool, feathers, hair, horns, hoof cuts and raw milk originating from live animals that did not show any signs of disease communicable through that product to humans or animals;

i. aquatic animals, and parts of such animals, except sea mammals, which did not show any signs of disease communicable to humans or animals;

j. animal by-products from aquatic animals originating from establishments or plants manufacturing products for human consumption;

k. the following material originating from animals which did not show any signs of disease communicable through that material to humans or animals:

i. shells from shellfish with soft tissue or flesh;

ii. the following originating from terrestrial animals: — hatchery by-products, — eggs, — egg by-products, including egg shells,

iii. day-old chicks killed for commercial reasons;

l. aquatic and terrestrial invertebrates other than species pathogenic to humans or animals;

m. animals and parts thereof of the zoological orders of Rodentia and Lagomorpha, except Category 1 material as referred to in Article 8(a)(iii), (iv) and (v) and Category 2 material as referred to in Article 9(a) to (g);

n. hides and skins, hooves, feathers, wool, horns, hair and fur originating from dead animals that did not show any signs of disease communicable through that product to humans or animals, other than those referred to in point (b) of this Article;

o. adipose tissue from animals which did not show any signs of disease communicable through that material to humans or animals, which were slaughtered in a slaughterhouse and which were considered fit for slaughter for human consumption following an ante-mortem inspection in accordance with Community legislation;
p. catering waste other than as referred to in Article 8(f).

The use of these fractions for different purposes is regulated by laws of individual countries, and in the EU for example, by common directives. The aim is to confine any microbiological problem or risk, and to prevent any harmful consequences. For example, animal wastes with a risk of agents of prion diseases need to be destroyed by forage.

The positive side of the waste or side stream treatment includes many potential processes for valuable products, also the microbiological ones. They are introduced elsewhere in this chapter and in this book. Such options include:

1. production of energy gases, such as hydrogen, methane or hytane
2. manufacturing of valuable biochemical from the wastes by biochemical and microbiological means
3. separation of fat or protein fractions for industrial uses, and their modifications
4. uses of microbial strains isolated from the surplus or discarded materials as bioprocess catalysts
5. use of hygienized and otherwise treated side streams for protein-rich animal feed
6. organic fertilizers for plant crop production and soil improvement

CONCLUSION

Regardless of the major risks of the spoilage or zoonotic microorganisms present in the meat or fish side streams, it is possible to utilize these substances and microbes in them for useful industrial production. At the same time it is of crucial importance to avoid the multiplication of any of the hygienic risks. Such protocols and processes have been developed by the meat and fisheries industries for the productions of safe animal feed, combustion for energy, gas production, biochemicals, important fractions for industries, and for organic fertilization and soil management. It is also possible to use the natural microbiota, as the rumen bacteria, safely for the production of important chemical products.

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