

## Harnessing the Microbiome for Protein Production

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**Received date:** March 16, 2021; **Accepted date:** April 13, 2021; **Published date:** July 07, 2021

**Citation:** Edward A. Calt, (2021) Harnessing the Microbiome for Protein Production. *J, Biotechnology and Bioprocessing* 2(6); DOI: 10.31579/2766-2314/034

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### Abstract

The microbiome of a ruminant animal rapidly converts cellulose into protein that is the true feed for the animal. Managed Ecosystem Fermentation (MEF) has harnessed this productive capacity into an industrial process. The MEF process rapidly converts cellulose, hemicellulose, carbohydrates, and starch into multiple products that have a wide range of applications in agriculture and industry. The protein produced has a complete amino acid profile and can be used as animal feed or for human consumption. Additionally, the protein contains lipids that contain Omega 3, 6 and 9, and other food additives and flavorings. The MEF process can convert approximately one metric ton of cellulosic waste per cubic meter of fermentation vessel per day into protein. This paper describes the process, results from the pilot plant, and compares the protein produced to fishmeal and other protein sources. MEF offers a technology to convert a significant amount of biomass into proteins, lipids, and enzymes in a way that is both economically and environmentally sustainable.

**Key Words:** managed ecosystem fermentation, rumen, ruminant, microbial protein, microbial lipids, microbial enzymes

Multiple microbiomes exist within nature, each codependent upon the others. Microbiomes are complex systems that are difficult to analyze as many of the microbes are codependent upon each other and as such cannot be isolated. However, this does not mean that an understanding of the process cannot be ascertained and/or controlled. For example, we do not fully understand the human microbiome, yet we are able to figure out what we can and cannot eat. And as such we are able to ascertain various parameters about which our own microbiome functions. With this understanding we are able to conduct our daily lives. If we view a microbiome from this perspective, then we can exert some control over it.

Microbiomes operate within a defined set of principles that dictate its behavior and hence its output. A microbiome must have an external source of food (carbon), energy, nutrients and water. There is an energy pyramid within the microbiome where several species consume the external material and generate a waste stream. In doing so, these microbes take the material with the highest energy density and break it down and then expel it as a waste that has a lower energy density. This waste stream becomes the food source for other microbes within the microbiome. This process repeats itself until there is a way to expel the lowest energy material produced from the microbiome. A basic law of nature,

which states no species can survive in its own waste stream, confirms this. These principals provide the basis for the industrializing a specific microbiome.

Both natural and managed microbiomes share several common traits. First, microbiomes are symbiotic. Each microbe is dependent upon its relationships with other microbes as well as its host environment. Second, natural microbiomes do not require a sterilized food source. Humans and animals do not require a sterilized food source. Their digestive microbiomes have evolved to protect the host. Essentially, most pathogens find an adversarial microbe within the ecosystem to consume it. Microbiomes have a wide range of materials that can be used as a food source. This permits them to survive for years at a time and is illustrated by the variety of food we eat. Third, microbiomes are focused on their own survival. This means they will adapt to changes in the environment in a way that ensures its survival. Finally, a microbiome works because a multispecies system has more chemical pathways to breakdown the biomass that any single species.

The microbiome in most animals simply break down the food so as to enable the subsequent utilization within the animal. The microbiome of a ruminant animal is unique in that it converts

cellulosic biomass into the food (i.e. protein, enzymes, lipids, and volatile fatty acids) for its host. This microbiome employs over 3,000 species of bacteria, yeast, fungi, and protozoa<sup>i</sup>. This consortium of microbes converts cellulosic biomass into the protein, amino acids, lipids and volatile fatty acids that are the true food for the host animal. This consortium of microbes is able to efficiently convert cellulose into protein. This efficiency is derived from the microbe's ability to generate over 30,000<sup>ii</sup> enzymes that facilitate the decomposition in an energy efficient manner. This microbiome was designed by nature to rapidly convert cellulosic biomass into protein for the animal.

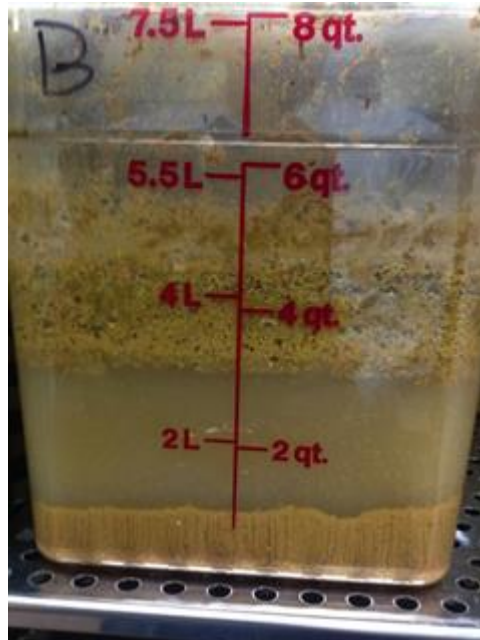
The ruminant microbiome conforms to the basics of microbiology. First, all cells require a source of carbon, nitrogen, phosphorous, and a variety of inorganic ions. Second, if there is a sufficient amount of nutrients available, growth and reproduction occur. Under optimal conditions, the microbes can double in number every 20 to 30 minutes. Third, microbes derive their energy in various ways. Some use oxidation or reduction of inorganic compounds. Others use the energy from the carbon compounds that are available. Finally, microorganisms are extremely adaptable to changes in their environment and food supply. Changes in the environment or food supply may cause the entire microbiome to change within one generation in order to survive. These elements provide the basis for the industrialization of the ruminant microbiome.

The ability of a ruminant's microbiome to rapidly produce protein has been studied in detail by the beef and dairy industries to ascertain how to put more meat on the bone and/or get more milk from the cow. This is the same process which Integrated BioChem, LLC has industrialized. There are differences between what has been studied and the Managed Ecosystem Fermentation (MEF) process. First, the MEF process has multiple levers that can be managed to change the composition of the protein and lipids produced.

Second, the microbiome of the ruminant changes once it is freed from the control systems inherent with the ruminant animal. This enables the implementation of methods that enable the management of the process. Third, unlike most industrialized bioprocesses, MEF is a very adaptive system that can deal with changes in its food, environment, and handling. Essentially, it is an adaptive system.

Within the rumen organ the cellulosic matter is continually mixed. This mixing serves several functions. First, it moves the digested matter and microbial protein produced on through the digestive tract. Second, it facilitates the transference of the microbes on to the new feed. This enables a continuous system. This also enables the expelling of the non-digestible matter.

In the MEF process, advantage is taken from the unique property of the process to self-separate. This enables the ease of mixing and separation. In Figure 1 this separation can be seen.



**Figure 1.** *Managed Ecosystem Fermentation*

Most of the biological activity occurs at the interface of the top mat and middle layer. There is a continuous circulation between the bottom and top mat. This recirculation is the exchange of nutrients and undigested matter between the various microbes.

After the microbes have had sufficient time to consume the feedstock, the protein that is in the bottom is harvested.

On an industrial basis the production equipment would look something like Figure 2, which is the MEF process run at a pilot

plant scale. The MEF pilot plant was run using 2 200-liter reactors. In this operation the process had a cycle time of 24 hours. In full-scale production the cycle time would be about 8 hours. This

means a reactor would be harvested 3 times per day. There would be a number of fermentation vessels. The number of vessels would be determined by the availability and digestibility of the feedstock.



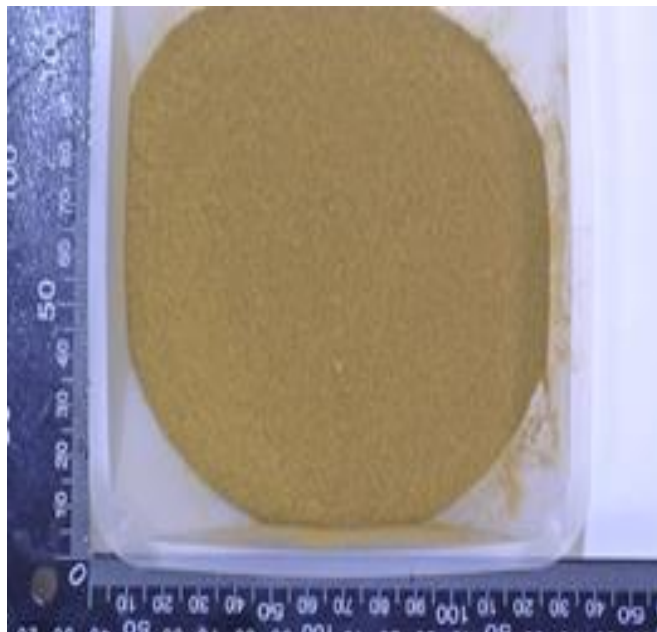
**Figure 2.** *MEF Pilot Plant*

The MEF process is capable of processing about 1 metric ton of food waste per cubic meter of fermenter per day. The current design specifications call for fermentation vessels with 5 cubic meters of fermentation capacity. The available inbound tonnage of feedstock would be divided by 5 to ascertain the number of fermentation vessels needed for the process for a particular site.

The processing of food waste using the MEF process is consistent with that seen in the ruminant animal. On a dry matter basis, about

50% of the biomass will be converted into methane (30%) and carbon dioxide (20%). These gases are reused within the process. About 25% will come out as a protein and the remaining balance will be composed of volatile fatty acids and non-digestible matter.

From this process the protein is extracted and dried. It is pictured in Figure 3.



**Figure 3.** *MEF Protein*

The protein is a dry, stable, sterile powder that can be incorporated into animal feed or be denatured for other industrial applications.

Raw MEF protein is the material that would be produced from the fermentation process after drying. Denatured MEF protein has had some of the lipids extracted from the raw MEF protein using a solvent that is used in food processing today. The numbers

provided for the raw MEF and denatured protein are from an Independent Laboratory<sup>iii</sup>.

The protein produced is comparable to fish caught in the ocean and used to produce fishmeal. Table 1 shows the results of the protein produced from the pilot plant as compared to open ocean fish.

Species		% Protein	% Fat
Anchovies		18	6
Herring (winter)		18	11
Herring (spring)		18	8
Pilchard		18	9
Mackerel (autumn)		15	27
Mackerel (spring)		18	6
Horse mackerel		16	17
Capelin		14	10
Blue whiting		15	2
San eel		18	7
Sprat		15	8
<b>Average</b>		<b>16.6</b>	<b>10.1</b>
<b>Raw MEF Protein</b>	Laboratory	26.00	31.36
	Pilot Plant	18.60	8.95

**Table 1** Raw Fish vs MEF Protein

The primary reason for the fat content being lower in the pilot plant is the inbound temperature of the feedstock was too low. This meant the microbes needed to consume the fat/lipids that would normally have been stored in the microbial cell.

These results suggest that microbial protein can be used as an alternative to fishmeal. When comparing the raw fish to fishmeal

it is necessary to understand how fishmeal is produced. The raw fish are cooked, ground, and then pressed to extract the fish oil. This leaves a higher protein content with less of the various fatty acids. This is a semi-denatured product, which can be compared to the MEF protein that has been denatured using a solvent that is used in the food industry. This comparison is shown in Table 2.

Table 2			
Fishmeal <sup>iv</sup> Vs Denatured MEF Protein			
Description	Average %	Minimum %	Maximum %
<b>Protein Content</b>			
Fishmeal	72	68	74
MEF	61.48		
<b>Oil &amp; Fat Content</b>			
Fishmeal	8	5	10
MEF	13.81		
<b>Ash Content</b>			
Fishmeal	7	4	10
MEF	6.14		
<b>Moisture Content</b>			
Fishmeal	7	4	10
MEF	3.27		

**Table 2** illustrates that the MEF protein is comparable to fishmeal. It is only how the denaturing process is managed that dictates the protein and fat content in the dry powder. In the above sample it contained about 14% oil and fat, which lowered the protein content on a mass balance basis.

Additionally, the MEF protein compares similarly to other animal based protein as shown in Table 3. Tables 1, 2, and 3 illustrate

both the range of values but also show that the MEF protein can be altered to meet the specifications desired for commercial purposes.

Source	Percent Per 100g of Meat			Kcal per 100 g
	Water	Protein	Total Lipid	
Goat	68.21	27.10	3.03	143
Lamb	57.48	25.55	16.48	258
Pork	60.66	29.41	9.44	211
Beef	64.46	27.42	7.72	187
Veal	67.01	28.07	3.39	150
Chicken	62.10	23.97	13.39	233
<b>MEF Protein</b>				
Laboratory	14.75	26.00	31.36	512
Pilot Plant	9.27	22.85	7.19	363
Denatured	3.27	61.48	13.81	515

**Nutritional Comparison**

**Table 3.** MEF vs Mammal & Fowl

Some of the lipids produced are shown below in Table 4 along with their industrial applications.

Description	Applications
Palmitic acid	Soaps, cosmetics and mold release agents
Stearic acid	Soaps, cosmetics, detergents, lubricants, softening and release agents
Oleric acid	Form of Omega 9 food additive, emulsifying agent, emollient, pharmaceuticals
Linoleic acid	Form of Omega 6 food additive, quick-drying oil, paint, varnish, surfactant, cosmetics
$\alpha$ -Linolenic acid	Pharmaceutical
Gamma Linolenic acid	Form of Omega 6 food additive, pharmaceutical
Stearidonic acid	Form of Omega 3 food additive, pharmaceutical
Docosatetraenoic acid	Form of Omega 6 food additive, pharmaceutical
Docosapentaenoic acid n3	Form of Omega 3 food additive, pharmaceutical
Docosahexaenoic acid	Form of Omega 3 food additive
Ceramide	Soaps, cosmetics and mold release agents

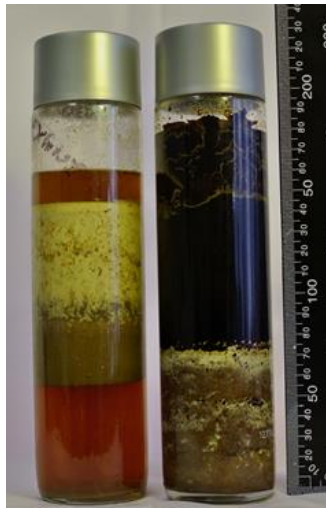
**Table 4.** Lipids Extracted From MEF Protein

In commercial production the fish oil is sold either for human consumption or reincorporated into the animal feed to a desired level. In the MEF process these oils are extracted using the same solvents that are used in processing soybean and vegetable oils.

The results from the separation process are seen in Figure 4. The difference between the 2 separations is the solvent used.

While Table 4 shows the commercial application, from a nutrition perspective the critical compounds are the Omega fatty acids.





**Figure 4. MEF Lipids Separated**

One of the critical aspects of these lipids is they are self-separating. This means that with existing food processing technology the lipids can be separated and then recombined into any desired formulation. Of particular interest would be the

separation of the phospholipids that can be sold to the pharmaceutical or cosmetic industries.

These lipids are also common in other foods we consume that originate in a ruminant animal.

Cheese Type	C <sub>2.0</sub>	C <sub>4.0</sub>	C <sub>6.0</sub>	C <sub>8.0</sub>	C <sub>10.0</sub>	C <sub>12.0</sub>	C <sub>14.0</sub>	C <sub>16.0</sub>	C <sub>18.0</sub>	C <sub>18.1</sub>	C <sub>18.2</sub>	C <sub>18.3</sub>	Total
Parmesan		1055	451	243	440	439	1540	3896	1171	3471	123		13,697
Cheddar	476	952	143	175	159	571	952	1556	794	2841	635	238	9,492
	1587	952	191	159	175	619	746	1253	508	1476	413	175	8,254
	1270	794	111	111	48	238	397	619	270	667	206	111	4,842
Swiss		170	90	45	122	208	311	1904	1427 <sup>a</sup>				42,77
		345	21	25	53	88	267	930	119 <sup>a</sup>				2,926
Edam		60	8	9	14	47	39	122	57 <sup>a</sup>				356
Mozzarella		54	7	1	120	12	27	76	66 <sup>a</sup>				
Camembert		35	5	14	35	43	69	270	210 <sup>a</sup>				681
	208	101	52				448	1028		1421			
		361	287	160	225	298	622	1442	303	1043			
Roquefort		961	626	707	2280	1295	3185	6230	2241	6282	896		25,969
Port Salut		41	4	8	54	33	86	275	199 <sup>a</sup>				700
Limburger		1475	688	24	50	92	602	565	709 <sup>a</sup>				
Münster		163	102	66	154	206	704	2057	833	1412	58	504	

<sup>a</sup>C<sub>18.0</sub> congeners

**Table 5. Concentration (mg/kg cheese) of free fatty acids in some selected cheese varieties [6]**

The lipids produced from the MEF process are the same as those that provide the distinctive flavor to various cheeses. In Table 5 these lipids and volatile fatty acids are identified in blue.

Table 5 shows that the lipids in the milk originate in the microbiome within the rumen organ. Controlling the exposure to various gases can increase the lipid content in the protein.

“Bacteria are known to produce a range of microbial oils that have multiple applications in industry. The production of these microbial oils has not been limited by the lack of desire for the end products, but simply by the absence of an appropriate means of producing microorganisms on a sufficiently large scale”<sup>v</sup>. MEF now makes the production of microbial protein and lipids possible

using existing production equipment, facilities and feedstocks, at a profit needed to provide the required economic return.

In most settings, the MEF process will be housed in a building with an insulated fermentation chamber to maintain the temperature needed to sustain the process. The building, fermentation chamber, tanks pumps, separation, drying and bagging equipment are all fixed costs. This is where the largest cost element of the process will be incurred in the form of depreciation. The estimated cost of production of one metric ton of MEF protein is approximately \$600 or less. This assumes that the feedstock is either free or at nominal cost. In most cases the MEF processing facilities will be collocated with industries that have high volumes of waste cellulosic biomass. The biomass will

be pumped at no cost to the MEF process as the biomass generator receives the benefit of a reduced disposal cost. If the feedstock is a food waste, then the cost of nutrients per metric ton of feedstock is about \$2.00. The MEF process is automated to the extent that personnel will be required in three areas; the process control, inbound feedstock preparation, and packaging and shipping. The facilities are expected to operate 24 hours per day, 7 days per week. As the MEF protein is in a dry powdered form, the shipping cost to the buyer should be minimal.

The MEF process addresses to growing needs in both the developed and developing world. The need to dispose of the waste biomass we generate every day is the first. The second is the need for a new source of protein. The MEF process is a technology that enables the production of protein and lipids in an economical and sustainable manner.

### Conclusion

The MEF process has replicated the first stomach of a herbivore. In the animal, the microbial protein produced is subsequently digested and converted into the meat we see in the supermarket. This implies that the microbial protein produced by the process can be used as a substitute for protein derived from the flesh of animals. The data derived from the independent laboratory analysis as compared to other animal protein studies confirm that this position. This means by processing waste organic matter with the MEF process, a new sustainable source of protein for multiple applications is now available. Initial applications would include inclusion in animal feed, and biodegradable adhesives.

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In developing the MEF process a number of design/operating advantages were pointed out by plant managers from several local biotech companies. First, the process avoids all the cost associated with sterilization. Second, the protein is self-separating. Third, the process can rapidly adapt to changes in the feedstock. Finally, the MEF process provides a processing capacity that is not inherent in other anaerobic digestion processes. MEF can process about one metric ton of food waste per cubic meter of reactor per day. This is because the microbial culture doubles every twenty minutes.

In summary, the MEF process provides a new sustainable source of protein while simultaneously addressing the issues associated with organic waste disposal. Essentially, it converts a waste stream into a revenue stream of high value compounds that have application in multiple industries.

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