### Power to Gas to Protein

Protein at farm scale from feed compatible components

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### Summary

In Western society, and even more so in upcoming economies, the demand for animal protein has been increasing with income levels. An intensive animal husbandry has been established to meet this growing demand. Over the years, the process of animal protein production has been optimized, resulting in higher efficiencies and yields. Drawbacks of this intensification process are phenomena such as high environmental impact, greenhouse gas emissions and decreasing farmer margins.

The use of waste streams from animal production, such as ammonia,  $CO_2$  and organic residues for the on farm production of microbial protein, would be a major contribution to make agriculture more sustainable. Not only would this reduce direct emissions from the farm into the environment, but it would also replace soy imports, and thus reduce the farmers' dependency on increasing soy prices on the world market . Furthermore, the production of microbial protein is independent of seasonal or climatic conditions.

This study is an exploration and reality check of a novel concept. It investigates to which extent protein production by means of single cell protein (SCP) production using hydrogen gas (H<sub>2</sub>), oxygen gas (O<sub>2</sub>), carbon dioxide (CO<sub>2</sub>) and ammonia (NH<sub>3</sub>) is economically feasible. These substances are all available on the farm. The process uses off peak (redundant) green electricity which is converted by means of electrolysis of water to hydrogen gas (H<sub>2</sub>) and oxygen gas (O<sub>2</sub>). The nitrogen source can be either ammonia (NH<sub>4</sub>) from fertilizers or recovered ammonia from manure stripping processes. In this way the ammonium is cleared from any other substances in the manure. The carbon source, carbon dioxide (CO<sub>2</sub>), can be concentrated from fermentation gases (e.g. biogas) or from exhausted industrial air emissions. Hydrogen, oxygen, ammonia and carbon dioxide can be used as inorganic substrates for a mixed microbial community of chemolithoautotrophic bacteria, i.e. hydrogen-oxidizing bacteria. These bacteria are capable of producing protein-rich biomass at relatively high yields, which can be used as feed on the farm. Indeed, the technological concept offers the farmer the opportunity of (partial) self-supply of feed.

Avecom, a company located near Ghent, Belgium, has experience with the production of microbiomes, i.e. interlinked communities of collaborating micro-organisms. Recently, this company has performed experiments in which communities, including autotrophic hydrogen-oxidizing bacteria, were used to convert hydrogen into protein. The results of the lab scale tests were promising.

Calculations have been made for three cases, each for a farm with 5,000 pigs, i.e.:

- Entire supply in protein demand on farm,
- Half of supply in protein demand on farm,
- 5% of supply in protein demand on farm (producing PHB for weaning piglets).

The costs of the input materials and the operational costs have been estimated based on available data in literature as well as on Avecom's experience and market insights. In relation to the capital expenditures, an amortization of 10 years has been taken into account. The value of the output material has been estimated based on available data.

The output material contains approximately 70% protein and 20% PHB (Polyhydroxybutyrate). This PHB has special value for enhanced feeds, due to its prebiotic and antibiotic activity. The value of the protein has been estimated at a conservative 1,000 EUR/tonne for the pure protein (with a composition comparable to that of soy protein). The PHB value was fixed at 5,000 EUR/tonne PHB.

Calculations show that when the protein value equals the value of soy protein, the costs exceed the potential value. However, when the PHB value is also taken into account, the gap between costs and benefits diminishes considerably.

If the potential protein value would be closer to the value of fish meal (2,000 EUR/tonne fish meal protein), the process might become economically feasible. If the PHB content could be increased from 20% to approximately 27%, the process would also become interesting from an economic point of view.

Costwise, if a decrease in raw material costs of approximately 13% could be realized, the process would become of economic interest even with the used value assumptions and yields of protein (value of soy protein) and PHB. The main cost factor is the production of H2.

In conclusion, the process is not yet economically viable with the assumptions used. However, since the gap between costs and benefits is not so big, further research might offer perspectives. More in particular, the composition and value of the product (protein, PHB) should be assessed by experiments on a (semi-)practical scale.

## List of abbreviations and terms

CDW:	Cell Dry Weight
CO <sub>2</sub> :	Carbon Dioxide gas
COD:	Chemical Oxygen Demand
H <sub>2</sub> :	Hydrogen gas
MWh:	Megawatt hour, at cost of approximately 50 EUR/MWh (source: Energy
	prices and costs report, Commission Staff Working Document, European
	Commission, 2014)
NH4:	Ammonia
NH <sub>4</sub> -N:	Ammonia-Nitrogen, i.e. ammonia expressed per unit nitrogen. E.g. 1 kg $NH_4$
	is approximately 0.78 kg NH4-N
0 <sub>2</sub> :	Oxygen gas
P:	Phosphorus
PHB:	Polyhydroxybutyrate
SCP:	Single Cell Protein
Soy price:	Soy contains 50% protein. The value of soy is 500 EUR/tonne soy. The value
	of soy protein is 1,000 EUR/tonne soy protein. The value of 1,000 EUR/tonne
	protein is used as the standard protein value in this study.

### 1. Rationale

At present, several major trends are of increasing importance, such as resource efficiency, increasing edible protein demand, climate change abatement and storage alternatives for off peak (green) electricity. Moreover, it appears that these trends are strongly interlinked. In fact, one can compare their relationship to a complex ecosystem. To face these challenges, the investigation and implementation of emerging sustainable technologies is of the utmost importance.

In Western society, and increasingly in other parts of the world as well, animal protein consumption is high. To meet this demand, an intensive husbandry has been established. Over the years, the production process of animal protein has been optimized towards higher efficiencies and yields. This successful intensification process however has its drawbacks, such as high environmental impact, greenhouse gas emissions and decreasing farmer margins. Indeed, it needs to be noted that the farmers themselves are subject to price policies of feed producers on the one hand, and those of retailers on the other hand. This implies that the farmers' margins have been decreasing over the years, despite higher efficiencies and yields. One of the major reasons for this is the increase in feed costs. These costs make up for about 50-70% of the production costs (including labour costs) (60-70% (source: www.thepigsite.com); 49% for breeding, 52% for fattening, 64% for closed farms (source: Technische en economische resultaten van de varkenshouderij op basis van het LandbouwMonitoringsNetwerk)). Due to the increase in soy demand worldwide, feed prices have increased in recent years with a factor 35% from January 2007 to September 2014 (ILVO, personal communication) and it is expected that these prices will continue to increase the following decades.

It is clear that in order to control production costs, alternative feed sources under direct control of the farmer might be of the utmost importance. Within this framework, InnovatieNetwerk and Avecom will investigate the possibilities of alternative protein production at farm scale. In order to obtain a first realistic view of the possibilities of this novel technology, it was decided to focus on feed-compatible raw materials and on reuse of the protein as feed on the farm itself. As such, ethical and food safety concerns in relation to the use of recovery products as raw materials are short-circuited. Later within the process, the use of recovery raw materials instead of pristine raw materials can be investigated and developed.

Hence, this study has to be viewed as an exploration and reality check of a novel concept. It will be investigated to which extent protein production by means of single cell protein (SCP) production using hydrogen gas (H<sub>2</sub>), oxygen gas (O<sub>2</sub>), carbon dioxide (CO<sub>2</sub>) and ammonia (NH<sub>3</sub>) is economically feasible. The process uses off peak (green) electricity which is converted by means of electrolysis to hydrogen gas (H<sub>2</sub>) and oxygen gas (O<sub>2</sub>). The nitrogen source can be either feed-compatible ammonia (NH<sub>4</sub>), such as a commercial fertilizer or recovered ammonia from stripping processes from for example manure. The carbon source, carbon dioxide (CO<sub>2</sub>), can be concentrated from fermentation gases (for example biogas) or from exhausted industrial air emissions. Hydrogen, oxygen, ammonia and carbon dioxide can be used as inorganic substrates for a mixed microbial community of chemolithoautotrophic bacteria, also called hydrogen-oxidizing bacteria. These bacteria are capable of producing protein-rich biomass at relatively high yields, which can be reused as feed at the farm. In fact, this technological concept offers the farmer the opportunity to (partly) self-supply of feed. The overall scheme is presented in Figure 1.

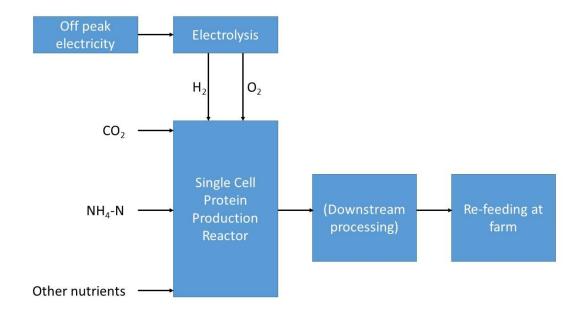


Figure 1: Overall scheme of Single Cell Protein production by using  $H_2/O_2$  produced by electrolysis of low cost respectively off peak (green) electricity.

## 2. Production mechanisms

In literature, there are many examples of stoichiometry for cell formation in autotrophic culture. *Ralstonia eutropha* is the most representative hydrogen-oxidizing bacteria, and its stoichiometry is expressed as follows (Cell growth and P(3HB) accumulation from CO<sub>2</sub> of a carbon monoxide-tolerant hydrogen-oxidizing bacterium, Ideonella sp. O-1, Tanaka et al., Appl Microbiol Biotechnol (2011) 92:1161–1169): 21.36 H<sub>2</sub> + 6.21 O<sub>2</sub> + 4.09 CO<sub>2</sub> + 0.76 NH<sub>3</sub>  $\rightarrow$  C<sub>4.09</sub>H<sub>7.13</sub>O<sub>1.89</sub>N<sub>0.79</sub> + 18.70 H<sub>2</sub>O

Hence, for the production of 1 tonne cell dry weight (CDW), the following quantities of raw materials are required at 100% assimilation of the raw materials:

- 440 kg H<sub>2</sub>
- 2047 kg O<sub>2</sub>
- 1853 kg CO<sub>2</sub>
- 110 kg NH<sub>4</sub>-N

In relation to phosphorus (P) and other minerals, it can be assumed that these only have a minimal impact on costs. For example, for the production of 1 tonne CDW, only 5 kg P are required. Therefore, P and other minerals will not be taken into account within the scope of this study.

Note that the above equation takes into account an assimilation of the raw materials of 100%. Based on the experience of Avecom and using a conservative estimation, a conversion efficiency of 80% will be used in further calculations. This implies that for the production of 1 tonne CDW, the following quantities are required:

- 550 kg  $H_2$  or 4,400 kg  $H_2$ -COD
- 2,047 kg O<sub>2</sub>
- 2,316 kg CO<sub>2</sub>
- 137 kg NH<sub>4</sub>-N

Now, 1 kg  $H_2$  corresponds to 8 kg COD- $H_2$  (COD, Chemical Oxygen Demand) since the reaction mechanisms is as follows:

H<sub>2</sub> + 1/2 O<sub>2</sub> = H<sub>2</sub>O, i.e. 1 kg H<sub>2</sub> (molecular mass: approximately 2 g/mol) needs 8 kg O<sub>2</sub> (molecular mass: approximately 32 g/mol) to be oxidized, hence 8 kg COD/kg H<sub>2</sub>

The yield of the process is:

1000 tonnes CDW x 0.8 conversion efficiency /(0.440 tonnes  $H_2 \times 8$ ) = 0.23.

This means that for every tonne COD- $H_2$  input, 0.23 tonne CDW can be produced.

Note that the above reaction relates to axenic culture conditions and that it can be assumed that a mixed culture could enhance the yield of the process to the range 0.25-0.30.

Literature reports a protein content of approximately 700 kg/tonne CDW. Hence, the following quantities of raw materials are required for the production of 1 tonne protein:

- 786 kg  $H_2$  or 6,288 kg  $H_2$ -COD
- 2,924 kg O<sub>2</sub>
- 3,309 kg CO<sub>2</sub>
- 196 kg NH<sub>4</sub>-N

In order to determine the value of this type of protein, Chapter 8 (Supplementary information) offers an overview of the composition of microbial protein (prutene), fishmeal and soy meal. In the economic evaluation in this study, the the microbial protein is valued on the same level as soy protein. This is a (very) conservative estimate, since the value of microbial protein is probably (much) higher.

Please note that in relation to protein production by autotrophic hydrogen oxidizing bacteria, literature reports numerous studies on axenic cultures (*Resource recovery from used water: The manufacturing abilities of hydrogen-oxidizing bacteria, Matassa et al., in review, 2015, water research, 68, 467-478*). Nevertheless, the promising yields from axenic processes, inherent axenic conditions and the utilisation of pure cultures, imply rather expensive protein production processes.

Avecom has experience with the production of microbiomes, i.e. interlinked communities of collaborating micro-organisms living in communities and as a community. The specific know-how of Avecom lies in the optimilazation of these microbiomes in relation to a certain goal. As such, Avecom has developed microbiomes for nitrification processes and soil clean-up processes which are subsequently produced and marketed. Recently, Avecom has performed experiments in which communities including autotrophic hydrogen oxidizing bacteria were used to convert hydrogen to protein. The lab scale tests showed promising results in relation to this process, including processes that are more economic while maintaining protein content, in relation to axenic production processes.

As an alternative to  $H_2$ , one could imagine that the best process for biomass production could be realized by using methanotrophs that are able to oxidize natural gas and produce SCP. This route of production of single cell has already been explored in the past (*Braude et al., 2007; Easthouse business solutions BV, 2005*). Yet compared to the yield of the hydrogenotrophic bacteria, the methanotrophs tend to have lower yield of the order of 0.10-0.15 (Sheehan and Johnson, 1971), which leads to lower biomass production. Also, in this specific case, the oxygen needed for their metabolism would have to be produced in an additional process. This would lead to more costs in comparison to the process of hydrogen production, where the oxygen is produced along with the electrolysis of hydrogen.

### 3. Input side: Raw materials and costs

#### 3.1 Hydrogen gas

- Electrolysis: At present, electrolysis can convert electricity to hydrogen and oxygen at 70% efficiency. Hence, 1 MWh produces 700 kWh chemical energy (H<sub>2</sub>), which is equivalent to 700/4 = 175 kg COD-H<sub>2</sub> (1 kg chemical energy in the form COD = 15 MJ = 4 kWh total (Energy recovery in wastewater treatment, Halim D., 2012., Report CE G7900, City College of New York Department of Civil Engineering).
- As a first preliminary estimate, one kg COD-H<sub>2</sub> costs 0.285 EUR/tonne (1 kg H<sub>2</sub> as such costs 2.28 EUR).
- According to the market prices of H<sub>2</sub>, 1 kg of H<sub>2</sub> costs 4 EUR (Roads 2 Hy Com Hydrogen and Fuel Cell Wiki, http://www.ika.rwth-aachen.de/r2h/index.php?title=Hydrogen\_Pathway:\_Cost\_Analysis&oldid=5029, 2014). This represents 8 kg COD. Hence, 1 kg of COD-H<sub>2</sub> costs 0.5 EUR.
- Overall, a cost of 0.4 EUR/kg COD-H<sub>2</sub> can be assumed. Hence, taking into account this cost and taking into account that 4,400 kg COD-H<sub>2</sub> is required for the production of 1 tonne CDW, the H<sub>2</sub>-cost is 1,760 EUR/tonne CDW or 2,514 EUR/tonne protein.

#### 3.2 Oxygen gas

Given the fact that oxygen is co-produced in the electrolysis process, plus the fact that it is not a limiting raw material in the thus produced quantities in relation to hydrogen gas, and given the fact that the solubility of oxygen is much higher compared to hydrogen gas, the cost for  $O_2$  is covered by the cost for  $H_2$ .

#### 3.3 Carbon dioxide

The costs for  $CO_2$  are at present 0.075 EUR/kg  $CO_2$  for for example application in greenhouses. However, when recovered from for example biogas, one could assume a cost of 0 EUR/kg  $CO_2$ .

In the worst case scenario of 0,075 EUR/kg CO<sub>2</sub> and taking into account that 2,316 kg CO<sub>2</sub> is required for the production of 1 tonne CDW, the CO<sub>2</sub>-cost is 174 EUR/tonne CDW or 249 EUR/tonne protein.

#### 3.4 Ammonia

- Pristine ammonia: The bulk cost for pristine ammonia is approximately 0.4 EUR/kg NH<sub>4</sub>-N. The gate cost is approximately 0.575 EUR/kg NH<sub>4</sub>-N. Hence, the ammonia cost per tonne CDW is 0.137 kg NH<sub>4</sub>-N/tonne CDW = 79 EUR/tonne CDW or 113 EUR/tonne protein.
- Recovered ammonia: The costs for recovered ammonia consist of (1) avoided treatment costs and (2) recovery costs. Ammonia in for example manure is often destroyed by nitrification/denitrification prior to discharge to the environment. In the first step of the process, the nitrification, micro-organisms convert ammonia to nitrate. In the second step of the process, micro-organisms convert nitrate to nitrogen gas. The treatment costs of this process are estimated at 4 EUR/kg NH<sub>4</sub>-N (Explorative research on innovative nitrogen recovery, Stowa, 2012, report 51).

Ammonia can be stripped from used resources by means of air stripping. This is a wellknown technique. In order to strip ammonium, a high pH is required (pH 10 to 12). In an air stripping process, the ammonium containing water is led through a stripping column in reverse flow through an air stream. The ammonia is transferred to the air stream, which is led to an absorber. The absorbed substance contains acid in which the ammonia dissolves and ammonium salts are formed. The ammonium salts are drained from the absorber while the ammonia-free air can be recycled to the stripping device. The costs for conventional air stripping are 1.9-3.2 EUR/kg  $NH_a$ -N with ammonium sulphate as recovered species (Explorative research on innovative nitrogen recovery, Stowa, 2012, report 51). Prior to air stripping, the solids in general need to be removed from the liquid. For most N-recovery technologies, a separation of the liquid fraction and the solid fraction of the used resources is required. The costs for the separation of 1 tonne DS by centrifuge are estimated at 65-99 EUR/tonne DS and by belt filter presses at 47-72 EUR/tonne DS (Marktconsultatie slibdroging- en slibontwatering, Stowa, report WO3, 2013). In the following cost estimation, an average cost of 75 EUR/tonne DS is taken into account. The separation costs per tonne NH<sub>4</sub>-N are presented in Table 1.

Used resource	DS content (kg DS/m <sup>3</sup> )	Concentration ammonium (kg NH <sub>4</sub> - N/m³)	Separation cost (EUR/kg NH <sub>4</sub> -N)
Pig Manure	54	3.23	1.254

Table 1: Cost estimation of separation process prior to ammonia recovery by ion exchange expressed as EUR/tonne  $NH_4$ -N.

The costs for regular air stripping are presented in Table 2.

Table 2:  $NH_4$ -N recovery costs by means of conventional air stripping.

Used resource	Air stripping costs (EUR/kg NH <sub>4</sub> -N)	Separation cost (EUR/kg NH <sub>4</sub> -N)	Overall recovery cost by means of air stripping (EUR/kg NH <sub>4</sub> -N)
Pig Manure	1.9-3.2	1.254	3.154-4.454

The costs for 1 kg recovered  $NH_4$ -N ranges from -0.846 EUR/kg NH4-N to 0.454 EUR/tonne  $NH_4$ -N. Hence the ammonia cost per tonne CDW is 0.137 kg NH4-N/tonne CDW = **-116 to 62 EUR/tonne CDW or -166 to 89 EUR/tonne protein**.

### 4. Reactor: Opex and Capex

Three cases are presented with regard to protein production on a farm with 5,000 pigs with a production yield of 0.23 tonne CDW/tonne COD-H<sub>2</sub>. Concerning the required protein quantity for 5.000 pigs, the following data need to be taken into account:

- Protein demand for 1 pig: approximately 45 kg
- Period: approximately 22 weeks
- Cycles/year: approximately 2.2 (including cleaning periods etcetera)

Hence, 5,000 pigs x 2.2 cycles/year x 0.045 tonnes protein: approximately 500 tonnes protein/year.

In the case of a pig farm of 5.000 pigs, the feed requirements are as follows:

- 100% protein supply: 500 tonnes/year,
- 50% protein supply: 250 tonnes/year,
- 5% protein supply: 25 tonnes/year (e.g high-quality protein for weaning piglets).

Based on Avecom's expertise, it is assumed that approximately 10 kg protein per m<sup>3</sup> of reactor per day can be produced. This means that the following reactor sizes (with related costs) would be needed:

- 100% protein supply: 137 m<sup>3</sup>
- 50% protein supply: 68 m<sup>3</sup>
- 5% protein supply: 7 m<sup>3</sup>

In relation to the investment costs (Capital Expenditures, Capex), the costs for the production reactor are estimated at 1,700 EUR/m<sup>3</sup> for 100-150 m<sup>3</sup> reactors, 2,000 EUR/m<sup>3</sup> for 50-100 m<sup>3</sup> reactors and 2,300 EUR/m<sup>3</sup> for 5-50 m<sup>3</sup> reactors. This is based on Avecom's experience and reactor related market insights. The operating costs (Operating Expenditures, Opex) are estimated at 100 EUR/tonne protein (143 EUR/tonne CDW).

Hence, the Capex and Opex for each case is:

- 100% protein supply: 137 m<sup>3</sup> at 232,900 EUR Capex and 50,000 EUR/year Opex,
- 50% protein supply: 68 m<sup>3</sup> at 137,000 EUR Capex and 25,000 EUR/year Opex,
- 5% protein supply: 7 m<sup>3</sup> at 16,100 EUR Capex and 2,500 EUR/year Opex.

Note that the electrolysis of water provides both the oxygen and the hydrogen. Both gases can be produced under pressure by electrolysis, and can thus be delivered to the hydrogenotrophic reactor. They do not need to be separated or purified, and can be

produced from brines. Overall, it should be possible to 'harvest' these reagents for hydrogenotrophic biomass production at low cost level.

### 5. Output side: protein and value

If the produced protein has the composition of that of soy protein, it is worth 1,000 EUR/tonne protein. Note that soy contains 50% protein! In fact, the value of soy is approximately 500 EUR/tonne. Hence expressed as protein, soy is worth 1,000 EUR/tonne soy-protein. This value will be used as default, i.e. as standard protein value. However, it can be assumed that the protein composition of the thus produced microbial biomass is of higher quality, comparable to that of fishmeal. This means that the protein would be worth around 2,000 EUR/tonne protein. Nevertheless, a conservative value of 1,000 EUR/tonne protein will be taken into account in this study.

Moreover, related to microbial protein production, it is known that without specific production conditions, the cells contain approximately 20% PHB (Polyhydroxybutyrate). This PHB is of special value for enhanced feeds. In fact, feeding studies in which PHB has been added as a feed ingredient have shown that it adds nutritional value to the diets of broiler chicks (US Patent 6,207,217). PHB also increased feed conversion values and/or induced prebiotic effects with various aquatic animals in aquaculture growth studies (*De Schryver et al.,(2010) Poly-β-hydroxybutyrate (PHB) increases growth performance and intestinal bacterial range-weighted richness in juvenile European sea bass. Dicentrarchus labrax. Applied Microbial Biotechnol 86: 1535-1541; The Nhan et al., (2010) The effect of poly β-hydroxybutyrate on larviculture of the giant freshwater prawn Macrobrachium rosenbergii. Aquaculture 302: 76-81; Sui L., Cai J., Sun H., Wille W., Bossier P. (2012) Effect of poly-b-hydroxybutyrate on Chinese mitten crab, Eriocheir sinensis, larvae challenged with pathogenic Vibrio anguillarum. Journal of Fish Diseases, 35: 359-364*).

Additional feeding studies have also been performed with sheep (Forni et al., (1999) Novel biodegradable plastics in sheep nutrition 2. Effects of NaOH pretreatment of poly(3hydroxybutyrate-co-3-hydroxyvalerate) on in vivo digestibility and on in vitro disappearance. J Anim Physiol Anim Nutr 81, 41-50; Forni et al., (1999b) Novel biodegradable plastics in sheep nutrition 1. Effects of untreated plastics on digestibility and metabolic energy and nitrogen utilization. J Anim Physiol Anim Nutr 81, 31-40) and pigs (Forni et al., (1999a) Digestive utilization of novel biodegradable plastic in growing pigs. Ann Zootech 48, 163-171). Thus, PHB is a promising ingredient for the production of enhanced feeds. The use of PHB as an alternative to antibiotics to control bacterial infections (Defoirdt et al., (2007) The bacterial storage compound poly-β-hydroxybutyrate protects Artemia franciscana from pathogenic Vibrio campbellii. Environmental Microbiology 9 (2): 445-452) and as a new biocontrol agent for sustainable animal production (Defoirdt et al., (2009) Short-chain fatty acids and poly-β-hydroxyalkanoates: (New) Biocontrol agents for a sustainable animal production. Biotechnology Advances, JBA-06216) has been discussed.

The value of PHB is estimated at 5,000 EUR/tonne. Hence, 1 tonne of CDW containing 0.2 tonne PHB, optimally gives an additional added value of 1,000 EUR/tonne CDW.

## 6. Overall economics

The following cases are presented:

- Case 1: 100% protein supply. All farm protein needs are supplied by means of the  $NH_{3}$ - $H_{2}$  production route,
- *Case 2*: 50% protein supply. Half of the farm protein needs are supplied by means of the NH<sub>3</sub>-H<sub>2</sub> production route,
- *Case 3*: 5% protein supply. Only 5% of the farm protein needs are supplied by means of the NH<sub>3</sub>-H<sub>2</sub> production route.

In relation to the benefits, it is assumed that the composition of the produced protein is comparable to the composition of standard protein. Furthermore, it is assumed that the produced cells contain 20% PHB. Hence, the value of the produced product on farm, consists of standard protein value and the PHB value.

By presenting three cases with other levels of self-supply in protein, other scenarios in discussion can be explored in collaboration with InnovatieNetwerk and partners in subsequent phases following this study. In fact, although the value of the protein share is at present estimated to be comparable to the value of standard protein, it might be of interest to verify the real composition and value of the produced protein, and more in particular the value of the thus produced products to, for example, weaning piglets. For this purpose, the different scales already have been elaborated. Moreover, PHB might be of interest with regard to fattening pigs and weaning pigs. If the partners and Avecom conclude that the PHB route needs further investigation, the different scenarios can be used to evaluate the effects of increased PHB yields.

Raw Materials	tonnes/year	EUR/tonne protein	EUR/year
H <sub>2</sub> -COD	3,144	2,514	1,257,000
CO <sub>2</sub>	1.654	249	124,500
NH4-N	98	100 (1)	9,800
CAPEX	EUR	Amortization	EUR/year
	232,900	10 years	23,290
OPEX		EUR/tonne protein	EUR/year
		100	50,000
TOTAL COSTS	tonnes protein/year	EUR/tonne protein	EUR/year
	500	2,929	1,464,590
TOTAL VALUE	tonnes protein/year	EUR/tonne protein	EUR/year
- Standard protein price	500	1,000	500,000
	tonnes PHB/year	EUR/tonne PHB	
- PHB	143	5,000	715,000
Total value: Standard protein price + PHB			1,215,000

Case 1. All protein is supplied by means of the NH<sub>3</sub>-H<sub>2</sub> production route, i.e. 500 tonnes protein/year (714 tonnes CDW/year).

(1) Average cost for  $NH_4$ -N of 100 EUR  $NH_4$ -N/tonne protein.

Based on the value estimation of the product and the cost estimations, it is clear that at present, the technology for protein production at farm scale by means of the  $NH_3$ - $H_2$  route is not yet of micro-economic interest. Note that the  $H_2$ -costs represent 86% of the total costs. All raw materials combined represent 95% of the total costs. Capex and Opex represent 5% of the total costs. If the costs for the raw materials could be decreased with approximately 175,000 EUR (about 13%), the process might be of economic interest. Another option would be to explore the real value of the thus produced biomass, since literature indicates that SCP might have a protein composition of higher value in comparison to the value of soy protein. Yet another option would be to increase PHB content of the biomass from 20% to 27% on dry matter basis.

Raw Materials	tonnes/year	EUR/tonne protein	EUR/year
H <sub>2</sub> -COD	1,572	2,514	628,500
CO <sub>2</sub>	827	249	62,250
NH4-N	49	100 (1)	4,900
CAPEX	EUR	Amortization	EUR/year
	123,500	10 years	12,350
OPEX		EUR/tonne protein	EUR/year
		100	25,000
TOTAL COSTS	tonnes protein/year	EUR/tonne protein	EUR/year
	250	2,932	733,000
TOTAL VALUE	tonnes protein/year	EUR/tonne protein	EUR/year
- Standard protein price	250	1,000	250,000
	tonnes PHB/year	EUR/tonne PHB	
- PHB	71	5,000	355,000
Total value: Standard protein price + PHB			605,000

# Case 2. Half of the farm protein needs are supplied by the NH<sub>3</sub>-H<sub>2</sub> route, i.e. 250 tonnes protein/year

(2) Average cost for  $NH_4$ -N of 100 EUR  $NH_4$ -N/tonne protein.

Similar to case 1, this second case, in which half of the protein demand at farm scale is provided by the NH<sub>3</sub>-H<sub>2</sub> route, is not based on profitable assumptions either. The raw material H2 makes up for approximately 86% of the total costs, and all raw materials combined represent approximately 95% of the costs. Again, the economics would become more attractive if the value of the produced product would be higher, if the PHB-content of the product could be increased and if the costs for raw materials could be decreased. In comparison to case 1, the Capex and Opex of case 2 are slightly lower, yet not to a significant extent.

	. ,		FUD /
Raw Materials	tonnes/year	EUR/tonne protein	EUR/year
H2-COD	157	2,514	62,850
CO <sub>2</sub>	83	249	6,225
NH4-N	5	100 (1)	490
CAPEX	EUR	Amortization	EUR/year
	16,100	10 years	1,610
OPEX		EUR/tonne protein	EUR/year
		100	2,500
TOTAL COSTS	tonnes protein/year	EUR/tonne protein	EUR/year
	25	2,947	73,675
TOTAL VALUE	tonnes protein/year	EUR/tonne protein	EUR/year
- Standard protein price	25	1,000	25,000
	tonnes PHB/year	EUR/tonne PHB	
- PHB	7	5,000	35,500
Total value: Standard protein price + PHB			60,500

Case 3. Only 5% of the farm protein needs are provided by the  $NH_3-H_2$  route, i.e. 25 tonnes protein/year.

(3) Average cost for  $NH_4$ -N of 100 EUR  $NH_4$ -N/tonne protein.

Similar conclusions can be made for case 3. For this third case it is however of special interest that the higher the value of the product, the more interesting a small reactor would become. If it appears that for example PHB would be of special interest to weaning piglets, the required PHB quantities could be provided by a small reactor. This would strongly decrease the required investments.

## 7. Conclusions

This study has explored the economic feasibility of a novel protein production at farm scale. The technical process converts off peak (green) electricity by means of electrolysis in  $H_2/O_2$ . The  $H_2/O_2$  is used together with ammonia and  $CO_2$  in a microbial production reactor to produce single cell proteins. Note that the cost estimations take into account the possibility to produce the single cell proteins from feed grade raw materials. Thus, possible (ethical/legal) objections and obstructions are avoided.

The costs of the input materials and the operational costs have been estimated based on available data in literature on the one hand and on Avecom's experience and market insights on the other hand.

In relation to the capital expenditures, an amortization of 10 years was taken into account.

The value of the output material has been estimated based on available data. The output material contains approximately 70% protein and 20% PHB. The value of the protein has been estimated at a conservative 1,000 EUR/tonne pure protein (with a composition comparable to that of soy protein, which is 50% of soy content). The PHB value was fixed at 5,000 EUR/tonne PHB.

Three cases have been studied, all for a farm with 5,000 pigs, i.e.:

- Entire supply in protein demand on farm
- Half of supply in protein demand on farm
- 5% of supply in protein demand on farm, which is of particular interest in relation to e.g. PHB for weaning piglets

The cost calculations show that when the protein value equals the value of standard protein, the costs exceed the potential value. When the PHB value is taken into account, the gap between costs and value is considerably smaller. However, based on the current data, the process is not of economic interest yet.

Thus, it would be of the utmost importance to have the market validate the real value of the product. Indeed, if the potential protein value appears to be closer to the value of fishmeal (2,000 EUR/tonne fishmeal protein), the process might become interesting. Moreover, from literature data, it appears to be possible to increase the PHB-content of the cells. PHB is in particular of interest with regard to decreasing the use of antibiotics, and might be of special interest to weaning piglets. If the PHB content could be increased

from 20% to approximately 27%, the process would become interesting from an economic point of view.

If the protein value would be comparable to that of fishmeal protein and/or if the PHB content could be increased, the NH<sub>3</sub>-H<sub>2</sub> production route might be interesting for entire self-supply of the protein demand on farm and PHB supply.

It might for example be interesting to further investigate the potential of PHB to decrease the use of antibiotics for weaning piglets. In that frame, a smaller scale reactor with limited investments might already be sufficient.

With regard to the costs, a decrease in raw material costs of approximately 13% would make the process interesting, even with the used value assumptions and yields of protein (value of standard protein) and PHB.

Although the process at present, with the used assumptions, is not yet of interest, further exploration is therefore strongly advised. In particular, the real composition and value of the product should be validated by the market.

# 8. Supplementary information: Animal/vegetable/microbial protein nutritional value comparison

According to the study of Taylor and Senior (1978), the main 'factors contributing to the nutritional value of SCP include protein content and digestibility, amino acid content, balance and biological availability. In addition, several other factors such as salt, carbohydrate, and lipid content and the presence of other components leading to improved or reduced nutritional value must be considered.'

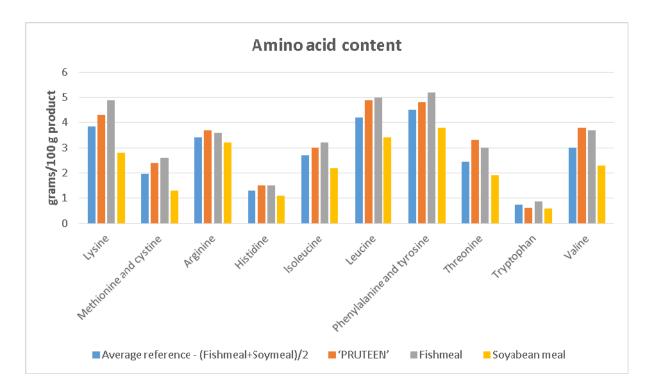
In the same study, fishmeal is referred to 'as the most valuable conventional high quality protein source for animal feeding'. Already during the late 1970s, SCP products were therefore competing with the fishmeal market, in view of the predicted 'fishmeal gap'. Of the many potential outlets, the use of SCP in high-quality compounded feeds for poultry and pigs was foreseen as predominant in the near future.

Table 3 compares the main parameter discriminating between animal protein (fishmeal), vegetable protein (soybean meal) and microbial protein (PRUTEEN) quality. The average between fishmeal and soybean meal was taken as reference for assessing whether the microbial protein (PRUTEEN) are closer to vegetable or animal protein.

grams/100 g product	Average reference - (Fishmeal+Soymeal)/2	'PRUTEEN'	Fishmea l	Soybean meal
Nitrogen	8.9	12.5	10.6	7.2
Crude protein N	55.6	78.1	66.2	45
Amino acid N	46.1	57.6	54.2	38
Crude fat	4.55	4.9	8.1	1
Lysine	3.85	4.3	4.9	2.8
Methionine and cystine	1.95	2.4	2.6	1.3
Arginine	3.4	3.7	3.6	3.2
Histidine	1.3	1.5	1.5	1.1
lsoleucine	2.7	3	3.2	2.2
Leucine	4.2	4.9	5	3.4
Phenylalanine and tyrosine	4.5	4.8	5.2	3.8
Threonine	2.45	3.3	3	1.9
Tryptophan	0.73	0.62	0.86	0.6
Valine	3	3.8	3.7	2.3

Table 3: Composition of high protein feedstuffs grams/100 g product (Taylor and Senior, 1978).

Figure 2: Amino acid content in gram per 100 g of product of animal, vegetable and microbial protein sources (elaborated from Table 3).



As figure 2 clearly shows, the amino acid content of SCP (PRUTEEN) is closer to that of fishmeal rather than to that of soybean meal.

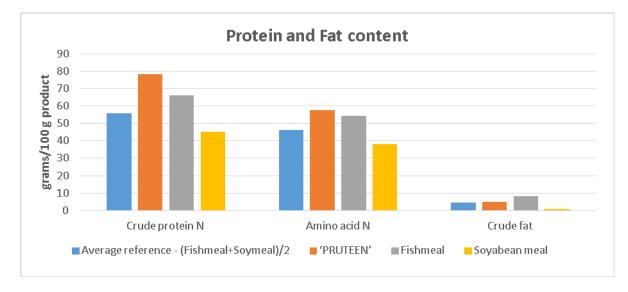


Figure 3: Protein N, amino acid N and crude fat content in gram per 100 g of product of animal, vegetable and microbial protein sources (elaborated from Table 3).

Figure 3 also confirms that SCP is more similar to fishmeal, having an even higher crude protein N and amino acid N content than fishmeal. Also, the crude fat content is comparable to that of fishmeal, whereas that content is negligible in soymeal. With regard to the protein digestibility and biological availability, the animal/vegetable/microbial comparison can be made taking as reference respectively casein, wheat grain and hydrogen bacteria.

Table 4 reports the protein availablity for proteolyc enzymes in vitro for pepsin and trypsin enzymes (*Volova and Barashkov, 2010*). The protein assimilation of hydrogen-oxidizing bacteria is clearly closer to casein, and it excels wheat protein assimilation.

Proteins availability for proteolyc enzymes in vitro (%)	Average reference - (Casein+Wheat grain)/2	Hydrogen bacteria	Casei n	Wheat grain
Pepsin proteolysis after 3h	34.5	39.6	44	25
Trypsin proteolysis after 6h	43.65	44	55	32.3

Table 4: Proteins availability for proteolytic enzymes in vitro.

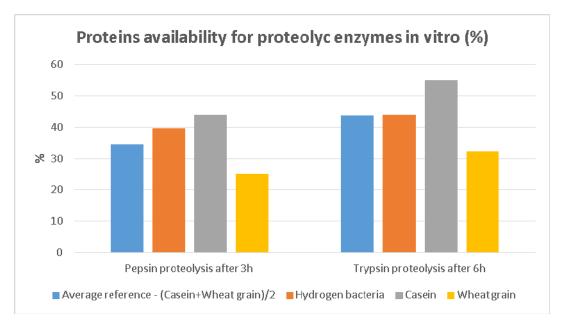


Figure 4: Proteins availability for proteolytic enzymes in vitro (elaborated from Table 4).

As Figure 4 shows, the digestibility of microbial protein is closer to casein, whereas vegetable protein are sensibly less digestible.

#### Conclusions

In conclusion, if we could demonstrate a similar trend in amino acid content and/or digestibility for the type of protein presented in this study, compared mainly to fishmeal and soymeal, these might represent good parameters for establishing the nutritional value of the microbial protein as closer to that of animal protein.

For further perspectives in our work, the concluding remarks of the study of Taylor and Senior (1978), are worth to be considered: '[...] the present products must be regarded as the first of many generations of continually improved ones. It can be predicted that second generation SCPs will be further processed to remove nucleic acid, thereby allowing greater direct human consumption. Third generation products may well be protein concentrates especially rich in specific nutrients. Assuming the predicted population increase and future protein demand figures are approximately correct, it would be unwise not to maintain effort into the exploration of such potential fields.'

### References

- Taylor, I.J., Senior, P.J. (1978). *Single cell proteins: a new source of animal feeds*. Endeavour, 2(1), 31-34.
- Volova, T.G., & Barashkov, V.A. (2010). *Characteristics of proteins synthesized by hydrogen-oxidizing microorganisms*. Applied Biochemistry and Microbiology, 46(6), 574–579.

### Samenvatting

Power to Gas to Protein: Protein at farm scale from feed compatible components Rik Daneels Msc (Avecom) InnovatieNetwerkrapport nr. 15.2.334, Utrecht, Mei 2015.

In de westerse samenleving, en zelfs nog meer in opkomende economieën, stijgt de vraag naar dierlijke eiwitten naarmate de inkomens stijgen. Om aan deze toenemende vraag te kunnen voldoen, is een intensieve veehouderij ontstaan. De productie van dierlijke eiwitten is geleidelijk steeds verder geoptimaliseerd om hogere opbrengsten en grotere efficiënties te behalen. Dit ging echter gepaard met een hogere milieubelasting, meer uitstoot van broeikasgasemissies en een dalende marge voor de boeren.

Het gebruik van afvalstromen van dierlijke productie – zoals stikstof, CO<sub>2</sub> en organisch afval voor de productie van eiwitten op de boerderij– zou een bijdrage kunnen leveren aan een duurzamere landbouw. Dit verkleint niet alleen de ecologische voetafdruk van de boerderij, maar het maakt de boeren minder afhankelijk maken van stijgende sojaprijzen op de wereldmarkt en de geopolitieke situatie in sojaproducerende landen. Bovendien is de productie van microbiële eiwitten niet seizoensgebonden of afhankelijk van klimaatomstandigheden.

De mogelijkheden van eiwitproductie op de boerderij met behulp van afvalstromen is onderzocht in deze studie. Hierbij is bekeken in welke mate eiwitproductie met behulp van *single cell protein* (SCP) op basis van waterstofgas (H<sub>2</sub>), zuurstofgas (O<sub>2</sub>), kooldioxide (CO<sub>2</sub>) en ammonium (NH<sub>3</sub>), economisch haalbaar is. Deze stoffen zijn beschikbaar (te maken) op de boerderij. Het proces maakt gebruik van *off peak* (overschot) groene elektriciteit die met behulp van elektrolyse van water wordt omgezet naar waterstofgas (H<sub>2</sub>) en zuurstofgas (O<sub>2</sub>). De benodigde stikstofbron kan bestaan uit ammonium (NH<sub>4</sub>) uit kunstmest, of ammonium verkregen via het strippen van mest. Bij dit strippen ontwijkt gasvormig ammoniak (NH3) uit de mest. De ammoniak wordt vervolgens via luchtwassers omgezet in ammonium. Via dit proces is de stikstof gezuiverd van ziektekiemen en andere ongewenste stoffen die voorkomen in mest. De koolstofbron, kooldioxide (CO<sub>2</sub>), kan worden geconcentreerd uit fermentatiegassen (bijvoorbeeld biogas) of uit industriële processen. Waterstof, zuurstof, ammonium en kooldioxide kunnen worden gebruikt als anorganische voedingsbodem voor een gemengde microbiële gemeenschap van chemolitho-autotrofe bacteriën, oftewel waterstofgasoxiderende bacteriën. Deze bacteriën zijn in staat om eiwitrijke biomassa te produceren met een relatief hoog rendement, die kan worden gebruikt als voer op de boerderij. Het technologisch concept biedt de boer mogelijkheden om (deels) zelfvoorzienend te zijn in voer.

Avecom, een bedrijf vlakbij Gent (België), heeft ervaring met de productie van microbiomen, oftewel onderling met elkaar verbonden gemeenschappen van samenwerkende micro-organismen. Dit bedrijf heeft recent experimenten uitgevoerd waarin microbiomen met o.a. autotrofe waterstofgasoxiderende bacteriën werden gebruikt om waterstof in eiwitten om te zetten. De resultaten van de laboratoriumtests waren veelbelovend.

In dit rapport zijn berekeningen gedaan voor drie cases, elk voor een boerderij met 5.000 varkens, te weten:

- Productie van de gehele proteïnebehoefte op de boerderij,
- Productie van de helft van de proteïnebehoefte op de boerderij,
- Productie van 5% van de proteïnebehoefte op de boerderij (PHB-productie voor biggen).

De kosten van de inputmaterialen en de operationele kosten zijn zowel geschat op basis van beschikbare data uit de literatuur, als op grond van de ervaring en het marktinzicht van Avecom. Wat betreft de kapitaalkosten is uitgegaan van een afschrijving van 10 jaar. De waarde van het outputmateriaal is geschat op basis van beschikbare data.

Het outputmateriaal bestaat uit circa 70% eiwit en 20% PHB (Polydydroxybutyrate). Deze PHB is van speciale waarde in veevoer, door haar prebiotische en antibiotische eigenschappen. De waarde van de eiwitten is voorzichtig geschat op 1.000 euro/ton eiwit (met een samenstelling die vergelijkbaar is met die van soja-eiwit,). De waarde van PHB is vastgesteld op 5.000 euro/ton.

De berekeningen geven aan dat wanneer de eiwitwaarde gelijk wordt gesteld aan de waarde van soja-eiwit, de kosten hoger zijn dan de opbrengsten. Wanneer echter de PHBwaarde ook wordt meegewogen, vermindert het verschil tussen kosten en opbrengsten aanzienlijk.

Indien de waarde van de eiwitten dichter bij de waarde van visvoer (2.000 euro/ton visvoerproteïne) ligt, zou het proces economisch haalbaar kunnen zijn. Indien de waarde van het eiwit gelijk is aan dat van soja-eiwit en het PHB-aandeel zou kunnen worden vergroot van 20% naar circa 27%, is het proces ook economisch haalbaar.

Ook wanneer we de productiekosten kunnen verlagen, bijv. door het verminderen van de kosten van de inputs met circa 13%, wordt het proces economisch aantrekkelijk. De grootste kostenpost is de productie van waterstof.

De conclusie uit deze studie is dat het proces op basis van de gebruikte veronderstellingen (nog) niet haalbaar is. Maar aangezien het verschil tussen kosten en opbrengsten niet groot is, zou verdergaand onderzoek wel degelijk nieuwe perspectieven kunnen bieden. Met name de samenstelling en de waarde van het product (eiwit, PHB) zouden moeten worden onderzocht op basis van experimenten op (semi-)praktijkschaal.