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Production of Fish Feed and Fish Oil from Waste Biomass Using Microorganisms: Overview of Methods Analyzing Resource Availability

Kriss SPALVINS[1]*, Dagnija BLUMBERGA[2]

[1,2] *Institute of Energy Systems and Environment, Riga Technical University, Azenes iela 12/1, Riga, LV-1048, Latvia*

Abstract **– Aquaculture is currently the fastest growing food-producing sector in the world. The growth of this industry has been rapid for the last 25 years, however, aquaculture still relies heavily on feed input from wild capture fisheries. Landings in wild capture fisheries have been stagnant for the last two decades; therefore, new alternatives for conventional fish meal and fish oil need to be found. In this review, various alternatives are described and their advantages and disadvantages are evaluated. Single cell oils (SCO) and single cell proteins (SCP) produced by microorganisms are recognized as the alternative with the most potential for replacing fish meal and fish oil in aquacultures. However, production costs of SCOs and SCPs are still higher than production costs of Omega-3 rich oils from other sources (wild capture, plant derived oils and genetically modified plants); therefore, currently used substrates need to be replaced with cheaper agriculture and industrial biomass residues applicable for microbial fermentation. In order to evaluate various biodegradable residues and find the most suitable ones for SCO and SCP production, methods analysing resource availability are reviewed.**

Keywords **– agricultural waste; aquaculture; by-products; fish meal; industrial waste; omega-3 oil; single cell oil; single cell protein**

1. INTRODUCTION

Growth of global aquaculture production has been rapid for the last couple of decades. According to data of the Food and Agriculture Organization (FAO) the average annual increase in production has been 7.1 % since 2000 [2]. In contrast, landings in wild capture fisheries are stagnating, showing no notable changes in total amounts of landings for the last two decades. Most recent data available from FAO shows that in 2016 global aquaculture production was 110.2 million tonnes, while landings from wild capture fisheries were 92 million tonnes (see Fig. 1(a)) [2]. Although production-wise aquaculture has surpassed wild capture fisheries, feed input in aquaculture still heavily depends on wild capture fisheries. In 2014, 16.9 % (15.8 million tonnes) of whole wild capture landings were reduced to fish meal and fish oil. Fish meal and fish oil are the most nutritious and most easily digestible feed ingredients for farmed fish [1], which is the main reason why approximately 70 % of all globally produced fish meal and fish oil is still used in aquacultures as feed (see Fig. 1(b, c)) [3]–[5].

* Corresponding author.
E-mail address: kriss.spalvins@rtu.lv

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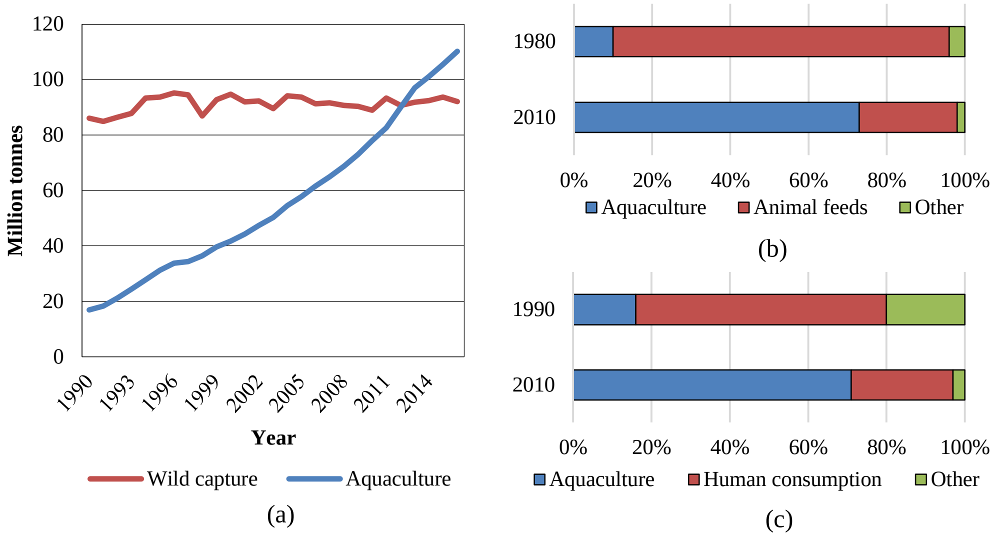

Fig. 1. (a) Change of global production volumes of aquacultures and wild capture fisheries [2]; (b) changing uses of fish meal [3]; (c) changing uses of fish oil [3].

2. Shortage of Fish Meal and Fish Oil

Fish meal and fish oil can be produced from many different species of fish, fish remains and other fish by-products, one of the main being the oily fish species of *Engraulis* genus of anchovies. Landings of anchovies are heavily affected by such events as El Niño phenomenon and fishing quotas implemented by governments in order to prevent overfishing [6]. Because of this, anchovies' landings have undergone great fluctuations over time [7].

Currently fish meal and fish oil prices have showed a rapid increase in the last years [1], for example, fish oil prices increased by 115 % in period 2011–2015 from USD 1300 to 2800 per ton [8], [9]. It is also important to note that in their latest report FAO expects a drop in prices in the period from 2016–2025 [1]. These fluctuations in price correspond with the irregular landings from wild capture for the most exploited fish species.

Main factors that affect fish oil market growth are aquaculture and human consumption, economic growth, increase in demand for healthier foods and growth of product distribution sectors. Due to these factors it is expected that the market of fish oil will increase by approximately 66 % in 2025 [1], [9], [10] when compared with the base period of 2013–2015. Although the market in general will grow, fish oil prices will decline by 14 % in nominal terms in the period 2016–2025 [1]. Estimates also predict similar changes in the fish meal market. Fish meal price reached its all-time high in 2013, it is expected that by 2025 the average price of fish meal will decrease by 3 % in nominal term estimates in comparison with the base period [1]. Future El Niño periods will cause fluctuations in fish meal and fish oil prices, but in general markets for these two products will grow considerably while price will somewhat decrease over time.

Since the amount of fish that is possible to be caught from wild capture fisheries is finite, so is the amount of fish meal and fish oil that is possible to be produced from these resources. While aquaculture production amounts are still growing, the shortage of these feed ingredients is causing fish meal and fish oil prices to increase [1].

Currently fish oil consumption in aquacultures is oscillating slightly below 800 000 tons and is expected to stabilize at 900 000 tons [4], [9]. This is due to competition between two major sectors of fish oil consumption – aquaculture and human nutrition and wider use of and substitution with oils extracted from microorganisms or plants. Although aquaculture is the main global consumer of fish oil today, consumption of fish oil by the aquaculture industry is estimated to increase by only 17 % in the period 2015–2025 [5], [8]. The main drive for growth of demand for fish oil will be due to its increased usage in human nutrition. It is estimated that amount of fish oil consumed by humans will increase by approximately 80 % in the same period [9].

Currently this problem is being solved by a shift of ingredient use from marine based to agriculture based feed ingredients [5], [11] and more selective use of fish meal and fish oil by using it in more specific stages of production such as hatchery and finishing diets and by using it to specific group of fish such as broodfish [1].

Using proteins and oils derived from plants in diets of captive fish is considered adequate in regard to their feed conversion ratio. However, doing so reduces the concentration of long-chain omega-3 fatty acids, such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) in the tissue of the fish. Another negative effect of switching feed source is the change of omega-6 to omega-3 ratio, where increased concentration of omega-6 fatty acids is paralleled with reduction of omega-3 [9]. Such change in ratio can be alarming when considering that high omega-6 to omega-3 ratio in diet coincide with multiple cardiovascular and neurodegenerative diseases, inflammation and cancer [9], [12], [13].

Although effective in the short term, these measures cannot solve the global shortage of fish meal and fish oil if aquaculture industry continues to grow at current projections – production being 38.8 % higher in 2025 than base period level of 2013–2015 [1]. It is expected that any increase in fish feed production will be coming from recycling of by-products [14] and alternative sources of poly unsaturated fatty acid (PUFA) rich products will be explored thoroughly in order to find other economically viable ways to satisfy increasing demand for PUFA rich oils.

Market growth of omega-3 rich oils is hampered, because aquaculture still requires fish oil that is derived from fish acquired from a production-wise stagnating industry of wild capture fisheries. The producers and exporters of fish oil are regularly constrained or experience conditions where a stable supply of raw materials for production is not maintainable. For example, in order to maintain the sustainability of anchovy fishing industry in Chile, the local government imposed fishing quotas based on assessments of anchovy populations [6], [9], [14]. In addition, warm phase in water surface temperature in central and east-central equatorial of the Pacific Ocean, also known as El Niño, reduced the available fish populations in South America [5], [8]. Therefore, the production of fish feed and fish oil from alternative sources is becoming a promising option for satisfying the increasing demand for these products [9].

The increasing demand for fish oil used either as fish feed or for human nutrition, has an essential impact on the environment, because the current production methods using animal-based raw materials cause strain on already scarcely available natural resources. Wild capture fishing has aggravated the state of several fish species, bringing some of them close to extinction [9], [15], [16]. Unsustainable demand, unpredictable landings and population imbalances not only make wild capture a less attractive option as source for raw materials, but are also raising significant concerns about the impact of extractive fishing on biodiversity of exploited habitats [17], [18]. Accordingly, the search for alternative sources of omega-3 rich oils and proteins applicable for use as fish feed should be one of the main priorities of fishing industry.

3. ALTERNATIVE SOURCES OF FISH FEED AND FISH OIL

Fish is acknowledged as one of the healthiest and most nutritious food products [1]; therefore, the growing demand for fish feed and omega-3 rich oils is stimulating the aspiration to find alternatives where these proteins and lipids can be produced. Currently aquaculture, agriculture, genetically modified plants and various microorganisms are considered as the most promising alternatives [9], [19].

3.1. Alternative Sources of Fish Feed

It is shown that fish, in regard to their nutritive requirements, are less flexible in diet composition than most land animals. A number of species of fish widely farmed in aquaculture are almost pure carnivores and they require high protein diets. These species of fish have very limited capability in utilizing carbohydrates as a source of energy. It is also believed that inclusion of certain types of carbohydrates in the diet of carnivorous fish can be detrimental to their health [20], [21].

FAO recognized the need for alternative sources of feed early on the development of aquaculture industry and listed alternative sources of proteins that could be used as partial or whole replacement for fish meal (see Table 1) [22].

TABLE 1. ALTERNATIVE SOURCES OF PROTEINS FOR FISH MEAL [22]

Plant	Animal	Microbial
Soy meal	Poultry by-products	Single-cell protein
Rapeseed meal	Feather meal	Single-cell oil
Sunflower meal	Shrimp and crab meal	Microscopic zooplankton
Oat groats	Blood flour	Phytoplankton
Cottonseed meal	Fish silage	Yeast
Wheat middlings	Meat meal	Bacteria
Grasses	Insect larvae	Algae
Leaf protein	Macroscopic zooplankton	Other
Vegetable silage		Recycled wastes

For feed to ensure optimal growth of the farmed fish it is important that protein content of the feed is in the range of about 15 % to 50 %. Of course, requirements differ from one species of fish to another and some vegetable derived sources lack in multiple amino acids such as tryptophane and methionine. It is also important for the diet to provide fish with proper balance in respect to proteins, carbohydrates, lipids, minerals, vitamins and other growth factors. Other factors such as composition, those affecting bio-availability, physical form, palatability and stability during storage should also be taken into consideration [22].

In some publications [23], [24] various options using industrial wastes as feed for microorganisms producing SCPs are reviewed. One of the most promising types of waste used for production of SCPs are residual streams from wood and straw based biorefineries such as spent sulfite liquor, spent sulphite liquor permeate, fiber sludge, hemicellulose hydrolysate etc.

Researchers from Sweden and Iceland managed to cultivate *P. variotii* and *F. venenatum* in spent sulphite liquor permeate and fibre sludge hydrolysate respectively [23]. They managed to acquire microorganism biomasses with protein contents as high as 51 % for *P. variotii* and 58 %

for *F. venenatum* of dry weight and did not find any significant levels of the most common mycotoxins in these products. After performing feeding experiments on fish they found out that fish fed with cultivated biomass content as high as 66 % and 68 % for *P. variotii* and *F. venenatum* respectively, did not show significant decrease in specific growth rate (SGR) or even showed increase in SGR in some feed mixes when compared to control group which was fed with conventional fish meal.

Experiments like these show the huge potential of microorganism produced aquaculture feeds from waste. Using waste materials as main feed input for microorganisms helps to reduce production costs for these fish feeds and also aids other industries in waste management as in this example for biorefineries. Most of the applicable by-products for SCP production have been reviewed before by Spalvins et al. [25], [26].

3.2. Alternative Sources of Fish Oil

It is possible to produce omega-3 fatty acids from various sources, of which soybeans, wheat, linseed, canola and microalgae have shown positive effects on growth on farmed fish when used as feed in aquacultures [4], [9]. Plants synthesize such fatty acids as linoleic acid (LA) and a-linolenic acid (ALA). These fatty acids are utilized in animals as precursors for the synthesis of PUFAs. This might suggest that sufficient LA and ALA dietary intake can provide with enough long chain PUFAs via endogenous synthesis. However, wild fish, while able to synthesize PUFAs in the same way as other animals, accumulate large amounts of omega-3 fatty acids by diet, not endogenous synthesis. This is because aquatic environment is rich in primary long chain PUFA producers, thus resulting in high PUFA concentrations within their tissue [9], [27]–[29]. Apart from that, non-genetically modified (non-GM) plants do not have the ability to synthesize such fatty acids as DHA or EPA [14]. For that purpose, genetically modified (GM) plants are used [30].

GM plants are already used as a fish oil substitute for farmed fish and human nutrition. Multiple studies have shown advancements in the development of EPA and DHA rich GM plants [30]–[33]. However, GM plants have lower DHA and EPA concentrations than those of oil producing microorganisms [9]. GM cultures have great potential in food production sector in general, but society still consider GM crops as unsafe [34]–[37] and in the European Union and other parts of the world growing, selling and importing of the GM crops face various levels of restrictions [38]–[41]. These restrictions make GM crops a less attractive alternative for fish oil. Advancements in microbial oil production technologies might provide with satisfactory amounts of PUFA rich oils [9].

Market for omega-3 rich products experienced rapid development when these fatty acids were recognized as vital for development of newborns and could be included in baby formulas [9], [42]–[44]. Since the 1970s technology of cultivating oil producing microorganisms has shown rapid development. Scientists found various fungi, bacteria, yeast and microscopic algae which were capable of accumulating more than 20 % of their dry biomass weight with oil [45]. These oils that were produced by using microorganisms were named single cell oils. Chemical and biochemical properties of these oils were similar to ones derived from plants and animals [46]–[49]. The main advantages of SCO production are high diversity, capability of accumulating large amounts of oil within the cells of cultivated microorganisms, fast growth of biomass in comparison with plants and animals and reduced costs of production [50]–[52]. In 2002 the first industrially produced SCOs were recognized as being safe by the US Food and Drug Administration and since then they are included in baby formulas for newborns, which in general has promoted increase in production volumes of these oils all over the world [9], [33], [45], [50]–[54].

Using microorganisms for production of SCOs rich in omega-3 can satisfy the growing demand for PUFAs and reduce the impact on the environment which is caused when these fatty acids are obtained from conventional sources [9]. This makes SCO production a promising alternative for fish oil.

3.3. Agro-industrial By-products and Waste as Microbial Feed

Apart from previously mentioned advantages, SCOs and SCPs can also be produced regardless of the weather conditions. While plants are affected by climate, microorganisms can be cultivated in controlled environments. Production technology also requires less space than plant based production and has higher production capacity and the highest productivity [9], [19], [51], [53], [55], [56]. Furthermore, production of SCOs and SCPs on a large scale has smaller impact on the environment and requires less space than conventional methods [9]. Available arable land areas cannot be increased any further [57], [58] due to depletion and erosion of the soil. This, in combination with growing human population [59], is noticeably increasing the demand for use of arable lands for production of direct foods only (harvested plant material which with minimal processing is ready to be used as food). Therefore, production of microbial oils and proteins is also recommended in regard to limited arable land areas that could be used for other cultures (cereals etc.).

Microorganism biomass processing technologies are well developed [9], but more research in substrate and microorganism selection could greatly improve production yields even further. It is assessed that, in microbial oil production, the carbon source adds up to 60–75 % of the total production costs [52]. Therefore, cheap carbon sources applicable for microbial fermentation need to be used in SCO production in order to make it an economically viable alternative [9], [51], [60]. For that reason, the most suitable carbon sources would be various agricultural and industrial residues. Finco et al. [9] divided these residues in four classes: (1) mono and disaccharides rich sources, such as molasses, industrial sugars and sugarcane processing residues; (2) starch rich sources, such as cereal and tuber processing residues; (3) glycerol residues from soybean, palm and tallow processing; (4) lignocelluloses rich sources from straw, sugarcane and wood processing and residues from cellulose production (biorefineries).

Use of biodegradable residues as substrate for the microbial fermentation can lower the production costs of SCOs and SCPs. Since there are many different kinds of wastes available, detailed availability analysis needs to be performed in order to find the most suitable ones. Availability analysis is further discussed in the next section.

4. METHODS ANALYSING RESOURCE AVAILABILITY

In order to analyse resources applicable for SCP or SCO production, it is necessary to carry out data analysis and assess total production volumes in specific regions or globally in general. It is also necessary to identify current usability of these resources, and to compare whether the current use of these resources is as effective as SCP or SCO production. If the use of the potential by-product has been less effective than using it in SCP or SCO production, more thorough analysis is required. Initial analysis of by-product volumes and usability in comparison to SCP has already been done by Spalvins et al. [25], [26].

However, such superficial analysis is inadequate and the fact that using the resource in the production of SCP or SCO would bring higher added value does not mean that the actual use of particular resource would be economically justified. Additional in-depth analysis is necessary in order to determine whether a potential resource would really be economically viable as a raw material for the production of SCP and SCO; therefore, it is necessary to perform an availability analysis for each resource, which addresses costs, local availability, transportation and required logistics systems. Consequently, in this chapter a number of biomass availability analysis models will be examined to find the most appropriate analytical solutions and factors that are relevant to the SCP and SCO production plant, actors involved in the whole supply chain and relevant at the national level as a whole.

In order to analyse in detail the availability of biomass and the conditions associated with its procurement, we will more closely review the biomass supply analysis on several levels: biomass supply chain between producer and processing plant (see Subchapter 4.1.); biomass supply chain between multiple producers, biomass logistics system (biomass transporters) and single processing plant (see Subchapter 4.2.); supply chain of multiple applicable biomass types on national level for multiple processing plants (see Subchapter 4.3.).

4.1. Availability Analysis between Biomass Producer and Processing Plant

At the first level of analysis, which is the biomass supply chain between single biomass producer and processing plant, it is essential to look at the factors affecting availability at a single producer level and examine how the supply of biomass from producer will affect the operation of processing plant. To review biomass availability analysis at this level, several supply chain models are available that cover the supply of various biomasses. Supply chain analysis has been done for resources applicable for SCP or SCO production such as cotton [61], sugar cane [62] and various cereal residues [63]–[69]. From the biomass models, the most comprehensive one at this level was the integrated biomass supply analysis and logistics model devised by Sokhansanj et al. [70]. This model is made for assessing collection of agricultural biomass that has complex logistics system. Since potential logistics systems for collection of other by-products (monosaccharaides/disaccharides, starch, protein/lipids rich by-products) are much simpler than for agricultural biomass [25], [26], by using elements of this model to analyse the production of SCP and SCO, the worst case scenario for the collection of resources is reviewed.

At this level of availability analysis, the following factors need to be considered: (1) availability period; (2) moisture content of the biomass; (3) weather factors that affect field operations; (4) properties and performance of the equipment; (5) dry matter loss; (6) cost identification [70].

The availability period is the first factor that needs to be evaluated before more thorough analysis of the supply chain can be done. While processing facility (fermentation plant) works throughout the year, many applicable resources are only available during specific periods. It is required to define factors for biomass availability period, since it will affect how available biomass is transported, stored and used in SCO and SCP production in order to fulfill the constant demand of biomass used in fermentation plant. The shutdown of fermentation plant due to feedstock shortage is costly, therefore storage and transportation of the biomass needs to meet the daily feedstock demand, which is directly affected by the availability period [71]. Some resources are available throughout the year (e.g. whey, spent grains etc.) [25] and for these storage and transportation logistics are simpler, since amount of generated biomass is used to select the optimal capacity of the fermentation plant. Whereas, for resources such as rice and wheat straw and corn stover, the biomass availability evaluation is much more complicated. For these resources, biomass availability is affected by various factors: (1) climate and weather affects moisture content of cobs and kernels, which changes the threshold when cereals become harvestable; (2) weather conditions also affect when harvest might be postponed due to unfavourable conditions; (3) harvesting habits of local farmers define how quickly grain harvest is completed, thus affecting when collection of straw or stover can begin [70].

For applicable biomass resources like cereal straw and stover it is important to evaluate all major factors affecting moisture content since that not only directly affects when biomass can be collected as it is in case of grain harvest, but it also affects dry matter loss, efficiency of used equipment and cost of collected biomass. Moisture content of straw or stover after grain harvest is affected by following factors: (1) temperature; (2) relative humidity; (3) wind velocity; (4) saturated water vapour pressure; (5) evaporation; (6) precipitation [70]. For other resources applicable for SCO or SCP production like glycerol, sulfite waste liquor, various food processing wastes, etc. moisture or water content is affected by applied production technology during which the particular by-product is generated [25], [26]. Resources with high moisture or water content usually start to spoil very quickly, therefore these by-products usually are not stored for prolonged periods of time or transported over large distances to conversion facilities. Similarly, as in case with moisture content of straw and stover, moisture or water content for these resources affects biomass loss and transportation costs.

Weather factors that affect field operations are relevant for resources which are collected from farmlands after harvests like cereal straw and stover, cotton stalk, fruit and vegetable waste and various other agricultural field wastes. Field operations are affected by the following weather factors: (1) average dry bulb temperature; (2) daily snowfall; (3) daily average relative humidity; (4) daily evaporation; (5) daily rainfall [70], [71]. In the model this data can be generated from hourly weather data of previous years in respective area. These factors in combination with specification of necessary equipment and labour are expressed in the model as amount of time during which field operation were suspended [70]. Resources that are generated as by-products from other processing plants do not require extensive field operations therefore these weather factors are mostly irrelevant.

Availability and price of all resources applicable for SCO and SCP production are also affected by properties and performance of equipment that is used for collecting, transporting and processing of relevant biomass. Equipment performance is defined as time that an operation takes to process a certain tonnage of material or perform certain tasks within the whole supply chain of the biomass [70]. Required tasks vary widely from one by-product to another. For example, collection and transporting for whey includes the following steps: (1) pumping of whey from dairy production plant’s storage tanks to transportation vehicle; (2) road transportation to fermentation plant; (3) weighting of the vehicle at fermentation plant; (4) unloading of whey into storage tanks or directly into bioreactor. On the other hand, wheat straw require a series of tasks: (1) in-field drying; (2) baling; (3) in-field transportation; (4) storing bales; (5) loading bales onto trucks; (6) road transportation; (7) weighing trucks at the fermentation plant; (8) unloading bales; (9) stacking bales at the plant storage site [71]. Depending on required tasks, the necessary equipment and required number of such equipment is determined, after which equipment performance can be assessed by analysing following factors: (1) covered area; (2) equipment speed; (3) equipment efficiency; (4) travel time; (5) load time; (6) unload time; (7) total transport time per load; (8) forward and return time of the transporter per load; (9) loading and unloading time per load; (10) efficiency factor for a transport equipment; (11) transport capacity; (12) volume of the container; (13) wet mass of the biomass; (14) wet bulk density; (15) time spent doing and time spent preparing for particular tasks; (16) equipment service life [70].

Biomass loses occur throughout the supply chain. If resource is plant material collected from farmlands then biomass is lost as fragile parts of the plant are broken off and lost during various operations. It has been previously assessed that such plant material loses more mass if moisture content decreases [70]. For other resources if stored or transported incorrectly biomass can be lost via uncontrolled fermentation and spoiling of the material. For production by-products like whey, sulfite waste liquor, fruit and vegetable waste, etc. spoilage is affected by water content, microbial pollution and time spent storing and transporting biomass to fermentation plant [25], [26]. Most of these factors are directly affected by applied technologies in production plant.

For cost identification, division of costs in fixed and variable costs is required. Fixed costs are the total costs of all taxes, housing, insurance and investments which are independent of equipment usage. Variable costs are total costs of repairs, maintenance, labour, fuel etc. associated with equipment use [70].

Detailed identification and assessment of factors affecting availability in the first level significantly facilitates further availability evaluation, since such model can be directly incorporated in next level of analysis, which is biomass supply chain between multiple biomass producers, hauling service provider and processing plant.

4.2. Availability Analysis between Multiple Biomass Producers, Hauling Service Provider and Processing Plant

Increasing the analysis level to several biomass producers will greatly increase the complexity of the overall supply chain model, as the conditions affecting availability are different for each biomass producer. The model is even more complicated due to the fact that, with the involvement of several biomass producers in supplying one processing plant, the model also requires the introduction of a hauling service provider and an optimization system that would ensure that the biomass flow is economically sound for all parties involved.

Modeling of supply chain which includes multiple by-product producers (farmers, industrial facilities, agricultural processing plants etc.), one or multiple hauling service providers and single SCO or SCP fermentation plant requires use of not only simulation model for particular by-products, but also use of optimization model. Optimization model needs to determine the following: (1) supply radius; (2) number of required by-product producers to secure yearly feedstock demand of fermentation plant; (3) required number, capacities and location of storage facilities; (4) number of required vehicles and equipment for each operation; (5) working schedule; (6) utilization rates [71]. Comprehensive simulation and optimization model devised by Ebadian et al. [71] was used as a methodical example at this level of availability analysis.

Since there are multiple approaches for how biomass is collected, stored and transported, various storage systems need to be evaluated. For production by-products generated in processing plants, complex transportation and storages systems are not required since most of the by-products start to deteriorate from the moment they are produced; therefore, if possible, applicable biomass is directly transported from production plant to fermentation plant. For these types of by-products expensive refrigerated storage facilities and transportation vehicles might be required, depending on supply area and travel distances to fermentation plant. If particular by-products (glycerol, molasses, sterile hydrolysates, dry production wastes etc.) [25], [26] have a longer shelf life, it is possible to store these wastes at production facility or at fermentation plant. For biomass which is collected from farmlands, more complex storage and transportation systems need to be evaluated, where it is possible to reconcile conflicting preferences between multiple by-product producers and hauling service providers. In order to optimize these preferences additional factors need to be introduced in the model next to the ones overviewed in the 1st level of availability analysis.

Following additional factors need to be considered: (1) number and volume of storage facilities (roadside storage and satellite storage systems); (2) equipment sharing among biomass producers.

To find optimal number and volume of used storage facilities various storage systems like roadside storage or satellite storage can be used [71]. With roadside storage system each biomass producer stores generated plant biomass at the roadside of the property. Due to short hauling distances this system is preferable to biomass producers, but also creates a large number of small storage facilities, which complicate transportation for hauling service providers. This system also decreases loading equipment utilization rates, due to small amounts of biomass that need to be loaded throughout the season [71], [72]. Satellite storage is more preferable to hauling service providers since intermediate storage is used between biomass producers and fermentation plant, but it also creates higher expenses for biomass producers, since producers have to cover longer hauling distances to storage sites compared to roadside storage [71], [73]. In satellite storage system storage facilities store biomass from multiple producers, therefore fewer storage sites need to be established and each storage facility has higher biomass availability, therefore hauling operations are more efficient [71]. In both storage systems, the number and location of storage sites depend on number of biomass producers and supply area. According to case study done by Ebadian et al. [71] using modified satellite storage system was up to 8.2 % cheaper than roadside system.

Equipment sharing among biomass producers is required since most of the equipment, if used for collecting biomass in only single farm, has very low utilization rate. Improved utilization rates via equipment sharing can significantly decrease fixed and variable costs associated with equipment use and make otherwise inefficient storage systems like roadside storage more viable [71].

4.3. Availability Analysis of Multiple Applicable Biomass Types on National Level for Multiple Processing Plants

Since lack of fish feeds rich in proteins and fatty acids is a problem on the global scale, it is necessary to look for resources that could be used for SCP and SCO production in large quantities in order to meet the huge demand; therefore, it is necessary to look at the availability of potentially suitable resources at national levels in order to see how much biomass can be collected and used in SCP and SCO production. Consequently, a model at this level of analysis needs to be reviewed as well. At the national level, availability analysis is significantly different from the other two levels of the biomass supply chain discussed here, since it is much more generalised.

In order to analyse overall applicability of the substrates, which in this case are various fermentable residues, it is important to overview publications where resource availability analysis at the national level was performed. In research done by Welfle et al. [74] biomass availability in the energy sector was assessed. Methods used in the said publication were based on supply chain model which analyses factors that affect resource availability. Since agricultural and industrial wastes are needed in microbial fermentation in order to make the production of SCO and SCP economically viable, in both industries (energy sector and SCO and SCP production) used biomass is biodegradable residues. Therefore, analysis of biomass availability in energy sector is not so different from analysis that needs to be done for the SCO and SCP production sector.

In order to analyse resource availability, the Biomass Resource Model (BRM) was used, which simulated all current systems that affect dynamics of biomass supply and distribution. BRM enabled to examine local resource availability until 2050. This model also allowed assessing the sensitivity of the biomass availability if changes in various supply systems took place.

Increased use of biomass in the national economy means that more intense biomass resource accumulation is required. Many EU countries are grounding their future strategies on availability of non-EU originated biomass resources. Welfle et al. [74] points out that countries should rethink their strategies and find better alternatives where more domestic originated biomass resources are utilized.

In their research Welfle et al. [74] found that household waste has the greatest primary bioenergy potential with plant cultures (energy crops) and agricultural residues following closely behind. This finding is also important for SCO and SCP production sector. This might push all dependant sectors, such as energy, SCO and SCP production etc. (all sectors which plan on expanding by increased use on household, agricultural or industrial residues or plant cultures), into forced redistribution of the available resources [75], [76].

Biomass waste resources were found to have the highest availability, with good potential in improving bioenergy sector. Resource availability can be easily affected by various factors, however, the most important ones are waste management strategies. BRM also allowed to define that waste biomass resources could be a very robust resource category that is not easily affected by other factors. This finding is also positive for the SCO and SCP production, because sustainable substrate source, such as waste biomass resources, are vital for development of the novel industry. With appropriate policies implemented, role of the waste biomass resources in bioenergy sector could greatly increase [74]. Under the right circumstances, that could cause competition for the resources between bioenergy and SCO and SCP production sectors in distant future.

BRM was controlled by set of supply chain drivers which were divided in following categories: (1) economic and development drivers, which include population changes (demand changes), resource distribution, technological development, productivity, GDP etc.; (2) infrastructure targets, which include supply chain development, system structure and production facilities (in regard to SCO and SCP production the later two are much more straightforward than in bioenergy sector); (3) physical and climate drivers, which include changes in climate, land use and water availability etc.; (4) food drivers, which include demand and consumption of food, diet change, change in productivity yields and calorie consumption (in regard to SCO production, if diet change increase omega-3 consumption from other sources demand for SCO would decline and vice versa); (5) resource mobilisation technical drivers, which include technological advances and industry residue generation; (6) resource demand drivers, which include resource use and demand by industry; (7) policy drivers, which include greenhouse gas emission targets, production efficiency and consumption targets, support policies and mechanisms [74]. For some of the mentioned drivers BRM allowed only partial analysis or were not analysed at all due to limits of the model itself and negligible effect some of these drivers had on the overall result. During development of availability model for residues applicable for microbial fermentation, these drivers need to be revaluated in regard to SCO and SCP production.

BRM model used in Welfle et al. [74] research was designed to perform analysis in three separate stages. In the first stage land area potentially suitable for energy crops were assessed. In SCO and SCP production this stage would require to assess the potentially suitable industries, which are or would produce residues applicable for fermentation. Thus, potential availability of biomass and its change could be determined.

In the second stage extent, availability and competing markets for various residues were quantified and forecasted. This stage would not require as extensive adaptation from one sector to another as it is required in the first stage, because second stage mostly works with data gathered from the initial stage.

In the third stage the production potential of SCOs and SCPs would be calculated. It would be done by using specific biomass quantities acquired from the second stage. In this stage pre-treatment and efficiency improving pathways would be considered as well. Production potential of SCOs and SCPs would be calculated by taking into account the conversion rations (grams of lipids or proteins produced from grams of consumed fermentable carbohydrates present in the residue) of each residue. After calculating specific conversion rations, available resources can be compared against each other.

5. CONCLUSIONS

Aquaculture is currently the fastest growing food producing sector in the world, however, further development of the aquaculture industry is threatened because still one of the main sources of feed for the farmed fish comes from resources acquired by wild capture fisheries (fish meal and fish oil). Landings of wild capture fisheries have been stagnant for the last 20 years and in the current situation wild capture fisheries cannot satisfy the demand of aquaculture with sufficient amounts of feed anymore. In order to secure the further development of aquaculture industry, it is necessary to find alternative sources of feed which would not create additional competition for limited resources (arable land, fish population in seas and oceans, primary plant based sugars etc.).

The most promising alternatives for conventionally produced fish meal and fish oil are agriculture, GM plants and various microorganisms. Agriculture products used in feeds for farmed fish have shown positive effect on growth. However, increased use of plant derived oils in fish diets can change omega-6 to omega-3 ratio in fish tissue, thus making such fish potentially harmful to the human health.

GM plants are already used as feed for farmed fish and their use in human nutrition is also expanding. Recent advancements in GM crops sector have already helped to develop EPA and DHA rich GM cultures. In general, GM cultures have great potential in the food production sector. However, the public still holds a very negative opinion on GM crops and there are many widely implemented restrictions on growing, selling and importing of these cultures. These obstacles make GM crops an unattractive alternative for fish feeds.

Cultivation technologies of proteins and oils producing microorganisms have shown rapid development in the last couple of decades. Current cultivation methods are matured and SCO and SCP production offer such advantages as higher production capacity, the highest productivity of all of the reviewed alternatives, high diversity and accumulation capabilities, fast biomass growth in comparison to other alternatives, reduced impact on environment, promoted industrial synergy by use of residues from other industries, decreased competition for limited resources (space, arable land, fish populations in world oceans) and capability of functioning regardless of weather conditions. This makes SCO and SCP production a promising alternative for fish meal and fish oil.

Further research in substrate and microorganism selection could greatly improve production yields of SCOs and SCPs. Carbon source is the main reason for increased production costs of SCOs and SCPs. Use of biodegradable residues as substrate for the microbial fermentation can lower the production costs and make SCO and SCP production economically viable alternative. Since there are many different wastes available, detailed availability analysis needs to be performed in order to find the most suitable ones.

In order to find the biodegradable waste substrates, which are applicable for cultivation of microorganisms, supply chain analysis through modeling is required. Potential substrate candidates need to be evaluated in regard to feed production potential, availability, competing markets, resource collection/harvest, changes in industrial activity, residue utilization, range of pre-treatment and energy conversion and conservation pathways. In future studies potential biomasses, such as food, cannery, straw, wood, husbandry, poultry, dairy and other agricultural and industrial processing wastes, residues from alcohol production and hydrocarbons need to be analysed using supply chain analysis.

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Kriss Spalvins, Ms. biol., is PhD student in Environmental Engineering at the Riga Technical University. He received Bachelor's degree of Natural Sciences in biology at University of Latvia in 2014 and Master's degree of the same specialty in 2016. Work experience includes: Researcher at Institute of Energy Systems and Environment located at Azenes iela 12/1, Riga, Latvia; Natural Science assistant (Internship) in University of Regensburg, Regensburg (Germany), Department of Cell Biology and Plant Biochemistry. He developed his Bachelor's thesis in Department of Plant Physiology (University of Latvia) where he worked on research of vermicompost effects on growth and development of various cultivated plants. During his internship in University of Regensburg he developed his Master's thesis where he researched defensin-like genes and ovule development of the flowering plant *Arabidopsis thaliana*. Current research focuses on use of biodegradable waste in cultivation of various oleaginous and protein rich microorganisms. ORCID: https://orcid.org/0000-0003-4464-1101

Dagnija Blumberga, Dr. habil. sc. ing., professor, director of the Institute of Energy Systems and Environment, Riga Technical University. Her two-step doctoral degree "Condensing Unit" was defended in Lithuanian Energy Institute, Kaunas (1988). Doctor Habilitus Thesis "Analysis of Energy Efficiency from Environmental, Economical and Management Aspects" was prepared in Royal Institute of Technology (KTH) Stockholm (1995) and was defended in Riga Technical University (1996).
Dagnija Blumberga has been part of academic staff of Riga Technical University since 1976 and director of Institute of Energy Systems and Environment since 1999.
The main research area is renewable energy resources. She has participated in different local and international projects related to energy and environment as well as an author of more than 200 publications and 14 books.
ORCID: https://orcid.org/0000-0002-9712-0804