AQUABIOPRO-*FIT* at a glance

PART III

Novel bioproducts development and validation in an operational environment

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Bio-based Industries
Consortium

PART III: Novel bioproducts development and validation in an operational environment

Summary:	Part III encompasses 4 courses that present the key concepts in the sustainable development of novel bioproducts from aquaculture and fisheries side-streams biomass and the validation of these bioproducts. This Part introduces the valorization of bio-waste by means of innovations and bioeconomy approaches in the light of the global efforts towards transition to a sustainable bioeconomy. Various bioproducts and processes for production of bio-based products are described, such as bioenergy, biomaterials, bioplastics, biopharmaceuticals, biocosmetics, biochemicals, bio-based food and feed ingredients. Emphasis is placed on the tremendous potential that the innovations in bio-sourced products, bioprocesses and other side-streams offer for adoption of clean technologies for the design and manufacture of innovative products in new and sustainable ways. Information is provided on the sustainability principles of economic development, which are grounded on strategies that outline the main pillars of the global policy towards a climate-neutral society by 2050. The strategies for increased public acceptance are focused on the economic and social sustainability dimensions of bioproducts development. Bioproducts (including those of fisheries and aquacultures) for human or veterinary use are subject to validation in an operational environment in order to ensure safety and quality standardization. The methods for extensive characterization of bioproducts are outlined, including the reference standards, quality specifications and strict process control measures that apply from early development through commercial-scale production. This Part also discusses some environmental hazards and risks associated with fisheries and aquaculture: biological, organic and chemical pollution, and habitat modification. The aspects of environmental impact assessment are covered, with a focus on methodologies for impact evaluation and regulatory aspects of environmental assessment.

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3.1.1 Introduction

Bioeconomy is another idea that formally came to light for conversation towards the start of the 21st century. As stated by the European Commission, "*The bioeconomy comprises those parts of the economy that use renewable biological resources from land and sea such as crops, forests, fish, animals and microorganisms to produce food, materials, and energy*." On the other hand, according to the Innovation Policy Platform, the improvement of bioeconomy and biotechnologies can prompt better stockpile and environmental sustainability of food, feed and fiber production, improving the water quality, provision of renewable energy, improving the wellbeing of animals and people, and helping in biodiversity protection by identifying invasive species. OECD (Organisation for Economic Cooperation and Development) defines the bioeconomy as "*the set of economic activities relating to the invention, development, production and use of biological products and processes*".

The general idea of bioeconomy is to develop energy, food and raw material supplies from renewable sources, moving away from our current dependency on fossil fuels and their derivatives. Bioeconomy can support sustainable development by improving the environmental efficiency of primary production and industrial processing and by helping to repair degraded oil and water.

Biobased economy is seen by numerous individuals as a future basis for a sustainable society and economy. New creative tools, associations, organisations and approaches are being created to help the biobased economy meaning to replace fossil based fuels and materials with sustainable biobased materials. Biomass, as a sustainable and bottomless resource, has many immediate and aberrant applications for food, feed, powers, fertilisers, chemical compounds and materials. The types of resources strongly differ per region. In the Mediterranean region, a lot of olive and vine biomass is accessible, in Scandinavia it is principally woods related biomass, while in numerous different regions there is a lot of horticultural biomass. However, biomass utilised for the biobased economy ought not compete with food production. In this manner, particularly biomass side streams are of great interest for the biobased economy. Agricultural and forestry service biomass side streams appear as residual stalks, straw, leaves, roots, desk, nut or seed shells, animal husbandry waste, forest harvest residues, saw mill deposits, and so forth. They are broadly accessible, sustainable, and cost effective. Their utilisation is carbon neutral, so such biomass can displace non-renewable energy sources, can assist with lowering GHG emissions while closing the carbon cycle and it very well may be changed over into a wide range of bioenergy and biomaterial products. When developing new paths for valorisation of biomass, it is essential to consider measurements and standards as far as "individuals, planet and profit" so as to make the change towards a sustainable future.

Innovations in bio-sourced products, bioprocesses and other by-products offer tremendous potential by encouraging the adoption of clean technologies that will

be used to design and manufacture innovative products in new and different ways.

3.1.2 Different Types of Bioproducts

Bioproducts are materials, chemical compounds and energy obtained from renewable biological sources. These incorporate agribusiness, forestry and organic waste among others. "Bioproducts" comprise a wide range of industrial and commercial goods that are described by a variety of properties, compositions and processes, just as many advantages and risks. Consequently, the expression "bioproducts" is a term that requires an assessment of the qualities of a specific item and the issues that arise on a case-by-case basis. There are several different types of bioproducts including:

Bioenergy

Bioenergy is produced in liquid, solid or gaseous forms when organic matter is treated using different physical, biochemical, thermochemical and other types of processes.

Bioethanol

Bioethanol is one of the most common bioproducts often blended with petroleum-based gasoline or diesel for transport or heating fuel. Typically, bioethanol is produced from the fermentation of various feedstocks that contain mainly fermentable sugars or carbohydrates. The available feedstocks for bioethanol can be categorised into four major types, as illustrated in Figure 3.1.1. However, corn and wheat grains are still the two most popular sources for ethanol production, though new technology is now producing ethanol directly from cellulose or syngas (a mixture of carbon monoxide and hydrogen) but this costs more than grain produced ethanol.

Biodiesel

Biodiesel has obtained increased interest as a nontoxic, biodegradable, and renewable diesel fuel. Being green energy, it could be a reasonable alternative for petroleum products. It can reduce air pollution by decreasing the levels of CO_2, SO_2, CO, and HC. The carbon cycle of biodiesel from biological resources is cost effective with respect to the photosynthetic process and burning emissions as a whole, especially because of the way that plants retain more CO_2 than those released by the biodiesel consuming procedure. Accordingly, utilising biodiesel can adequately decrease the release of CO_2, protect Earth ecosystems, and maintain the ecological balance, when compared with the utilisation of fossil fuels. The main concern with biodiesel fuel is its significant expense. It is normally created from vegetable oils, fats and oils in a procedure called transesterfication. Soybean and canola are examples of oilseed crops that can be utilised in biodiesel production just as reused vegetable oils from restaurants and fats from animal rendering (Figure 3.1.2). The modern creation of oil-carrying microalgae for the production of biodiesel is also in progress. Biodiesel

can be utilised in its pure form (B100) or mixed with petroleum diesel. Popular blends include B2 (2% biodiesel), B5, and B20.

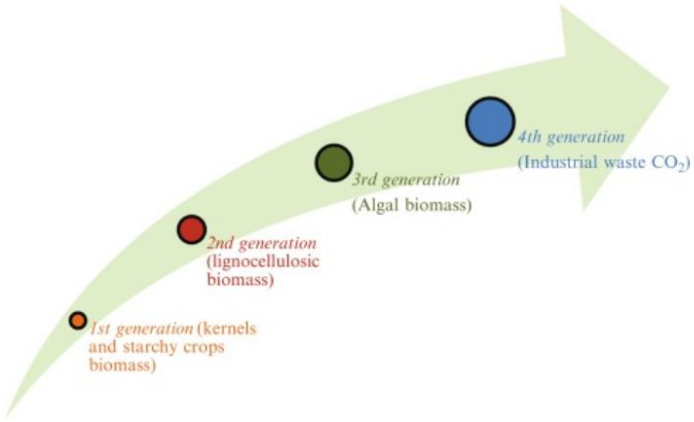

Figure 3.1.1 Bioethanol feedstocks classification

Figure 3.1.2 Soybean for biodiesel production (source: ADM plans to acquire Prairie Pride's soy crush and biodiesel facilities in Deerfield, Mo.; Photo: Prairie Pride Inc.).

Biogas

Biogas manufacturing is a well-known procedure for energy generation, nutrient recovery, and valorisation of natural deposits. It is produced from anaerobic digestion, a procedure wherein natural materials such as manure, rural harvest residues, dedicated energy crops and food processing residues are degraded by microorganisms, in anaerobic conditions to create methane for power and heat (Figure 3.1.3). Generally, the biogas comprises methane, carbon dioxide, and other contaminating substances. The elimination or transformation of CO_2 (biogas upgrading) and the purification from other impurities (biogas cleaning) are essential for improving the calorific value of biogas.

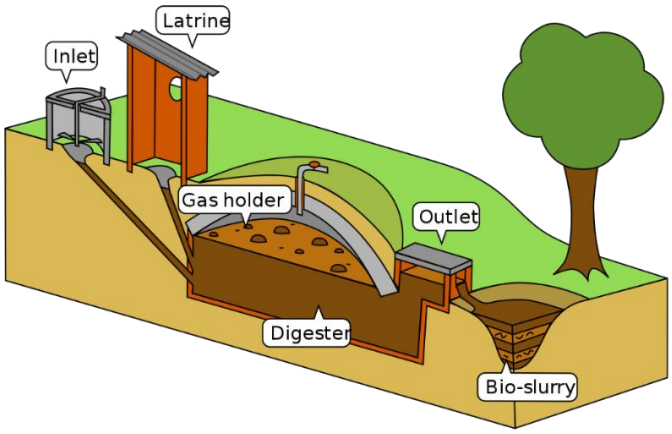

Figure 3.1.3 Biogas production scheme (source: https://globalinnovationpath.com/en/biogas-powering-china/) .

Biomass

Biomass is a complex renewable material with substantial chemical fluctuation (Figure 3.1.4). Its capacity for energy generation differs in regard to the method employed (from simple to highly sophisticated technologies). Since this natural resource can be found all over the world, its use for energy production and delivery of secondary products (solid, liquid, and gaseous fuels) should be reinforced thus improving the recovery capacities of agricultural, forestry, and industrial residues. Together with other renewable resources, such as solar, wind, and hydroelectric energy, biomass is a major future sustainable resource. Different crops such as switchgrass, agricultural crop residues (e.g. sunflowers), forestry and a wide range of organic matters can be burned directly or compressed into bioproducts such as briquettes and pellets for direct utilisation to produce heat and power. The most recent bioenergy technology comprises pyrolysis, gasification, solar-to-fuel and genetic manipulation of organisms to produce hydrocarbons.

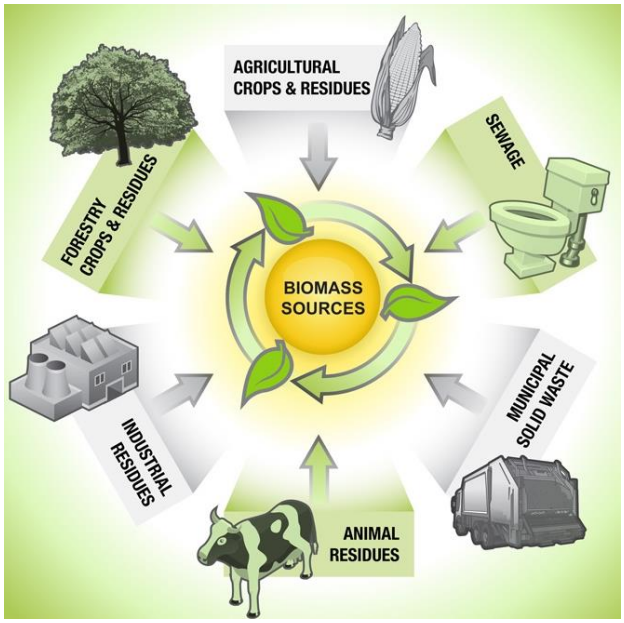

Figure 3.1.4 Biomass sources (source: https://www.cleantechloops.com/an-introduction-to-biomass-energy/).

Biomaterials

Late advances in natural fiber production, genetic engineering and composite research offer new prospects for development of improved materials derived from renewable resources. Thus enhanced support for global sustainability is achieved. Biofibres is one of them. They can be created and customised to meet different technical requirements. Biofibers are degradable, derived from natural sources as wood, agricultural crop residues and purpose-grown crops such as hemp and flax. Generally, they are used in furniture manufacturing, paper and textiles either all alone or mixed with petroleum-based polymers and resins to produce stronger, more solid biocomposite materials in the car, aviation and other manufacturing sectors.

Bioplastics

The term "bioplastic" means a plastic material that is based (wholly or in part) on organic biomass rather than petroleum. Generally, they are produced from starch crops (e.g., corn, potatoes, wheat) and vegetable oils (e.g., canola or soybeans) (Figure 3.1.5). Several types of bioplastics are known: starch-based, cellulose-based, protein-based, aliphatic polyesters, polyhydroxyalkanoates, bio-derived polyethylene, genetically modified feedstocks and lipid derived polymers. Bioplastics are biodegradable and are being adopted for a number of applications in automotive, electronics, food and beverage packaging, agriculture, textiles, health care, etc. Generally speaking, the greatest advantages of a growing bioplastic industry are a smaller energy footprint and less polluted ecosystems.

Figure 3.1.5 Corn for biofibers (source: https://www.biodeg.org/subjects-of-interest/bio-based-plastics/).

Biopharmaceuticals

Biopharmaceuticals, such as sugars, proteins, nucleic acids, living cells, or tissues, are medicinal products derived by extraction or semi-synthesised from natural sources like humans, animals, or microorganisms. Unique in relation to traditional pharmaceuticals produced by chemical synthesis, most biopharmaceutical items are obtained from biological processes including the extraction from living systems or the production by recombinant DNA technologies.

Some biopharmaceuticals are plant-derived, including some vaccines, antibiotics and drugs, which have therapeutic value. For example, producing insulin from safflower could significantly diminish its cost.

Figure 3.1.6 Bio-Carotene capsules by Pharma Nord (source: https://www.pharmanord.com/).

Biopharmaceuticals have multiple clinical applications and various advantages for disease therapy, prevention, and diagnosis.

Therapy

Biopharmaceuticals are used in different therapies such as recombinant, protein, antibody, cell and gene therapy. They effectively and safely cure or treat diseases. By demonstrating biological activity they perform specific functions and influence the disease pathophysiology. In comparison with chemical drugs, biopharmaceuticals are more complicated, have various routes of administration and different pharmacokinetics.

Prevention

Biopharmaceuticals can be used as vaccines for infectious disease prevention. They generally contain a biological agent that resembles a pathogenic antigen.

Diagnosis

Apart from therapy and prevention, some biopharmaceuticals can be applied in diseases diagnostics. For example, monoclonal antibodies have been successfully applied in the diagnosis of some cancers and infectious diseases, and more are being developed.

Biocosmetics

Over the years the use of cosmetics has taken a new course. This is due, on the one hand, to the better access to information and has also been driven by the consumer's interest on the origin, safety, and sustainability of the products they purchase. Another class of personal care products referred to as biocosmetics have emerged through advanced technologies, such as nano- and biotechnology. These products are safe for humans, obtained in harmony with the environment, utilising numerous industrial wastes as raw materials, thus creating high-valued products and eliminating a significant amount of pollutants. Vegetable oils, starches and proteins are utilised as main raw materials for the production of a range of different types of cosmetics including deodorants, shampoos, make-up, sunscreens, beauty and personal hygiene products (Figure 3.1.7). There is an expanding market interest for bioproducts, particularly to replace those currently made from petroleum-based sources.

Figure 3.1.7 Biocosmetics products (source: https://www.janssen-cosmetics.com/en/).

Biochemicals

Renewable chemicals are a new category of novel products. They are building blocks and ingredients for the production of chemicals, derived from renewable feedstock and sustainable advanced manufacturing processes, leaving a smaller environmental footprint than traditional petrochemicals and biobased chemicals. These products include biochemical intermediates and vegetable oils for sectors with higher value applications such as detergents, and biolubricants and

chemicals for the oil industry. Example products include bioplastics, biologically based solvents, surfactants and lubricants where biodegradability and the avoidance of harmful emissions are primary considerations. Vegetable oils from corn, soybean and canola are typical organic matter used for the manufacturing of chemicals such as solvents, lubricants and adhesives. They can also be acetic acid, succinic acid, glycerol and methanol that can be used in the manufacture of high-value, bioproducts.

The chemical companies of all sizes and at every stage in the production chain are looking to produce and supply more of their chemicals from plant-based feedstock. Growing new crops or plants for isolation of bioproducts and renewable chemicals offers a wide range of potential benefits:

- ✓ decreased reliance on imported oil and polymers
- ✓ lowered greenhouse gas emissions
- ✓ sequestration of carbon dioxide
- ✓ availability of complex structures produced by natural synthesis
- ✓ novel valuable products
- ✓ new industries
- ✓ better consumer acceptance of biobased products
- ✓ improved utilisation of land resources, especially in marginal farming areas and rangeland irrigation development areas.

These advantages, of course, assume price competitiveness and a chemical and physical profile that is at least comparable with synthetic chemicals. Final price of biochemicals depends on the price of raw material, and the cost and availability of technology for processing. Multiple renewable chemicals are extracted from existing irrigated crops (relevant to the rangelands). Among them the following can be listed.

- ✓ ***Sugar cane bagasse***: lingo-cellulosic biomass — such as waste from sugar cane — has been transformed into industrial sugars. These sugars are an essential component of biobased chemicals and fuels, including polypropylene and polyethylene.
- ✓ ***Avocados***: pits unused from producing guacamole are transformed into a polymer material. The material is 100% biodegradable and compostable, used as either a resin or an additive. Examples include biodegradable and compostable thermoplastic resins, and biodegradable additives, which can be blended with petroleum products for partial biodegradability.
- ✓ ***Tomato waste***: waste fiber from tomato processing (during the making of tomato sauce) has been employed to develop another type of bioplastic. This is part of a project to find more sustainable alternatives to petrochemical-based materials used in car manufacturing.
- ✓ ***Agricultural waste***: an ash by-product has been utilised to make stronger cement. Pre-treating a high-lignin ash by-product and adding it to cement made the cement stronger because the ash reacted chemically with the cement. After some testing, it was determined that using the agricultural residue ash to replace 20% of the cement by mass increased the strength of concrete by 32%.
- ✓ ***Vegetable oils***: Fats and oils are finding new potentially high volume industrial applications as transformer fluid. The best alternative already introduced in the US seems to be simple plant oil having a flash point of

330°C. The epoxidation of triglycerides, such as soy and linseed oil, is a well-known industrial process using hydrogen peroxide/formic acid. In industry, vegetable oil epoxides are used as PVC (polyvinyl chloride) stabilisers.

- ✓ ***Sugar and starch***: The most popular biobased plastic is polylactic acid (PLA), derived from sugar and starch. PLA has properties similar to those of conventional mass-produced thermoplastics and can be handled on existing production lines. Because it is compostable, PLA has significant potential for disposable packaging, such as beverage cups and plastic food packaging trays. One disadvantage of PLA is that its low melting point makes it unsuitable for items exposed to heat. Moreover, the chemical industry uses starch for producing surfactants, polyurethanes, resins and biodegradable plastics. In construction, starch is used for concrete mixtures, plasters and insulation. It is also used in metal and mineral processing, oil drilling, paper and board industries, cosmetics and health care, and through fermentation is turned into cyclodextrin, which is used as stabilisers and excipients in the pharmaceutical, cosmetics, food and agri-industries.
- ✓ ***Papaya and pineapple***: Economically important enzymes derived from papaya involve papain and chymopapain. They are used medicinally and as meat tenderisers. Bromelain — a protein-digesting and milk-clotting enzyme from pineapple juice — and malt extract (a product from barley that contains amylolytic enzymes) are other examples.
- ✓ ***Plant-based starches and proteins***: Besides the increased demand for natural confectionery, savory and sweets, there are also growing concerns over non-halal gelling agents, such as gelatin, in Muslin cultures. Many manufacturers are already shifting to plant-based starches and proteins to replace these non-halal ingredients.

Bio-based food and feed ingredients

Bio-based food and feed ingredients are the products used in food and feed industry which could be produced by any biotechnology process. These compounds could be from microbial, animal or plant sources, but also food wastes and by-products from food and feed industries. The latter are potential sources to find different molecules and develop new ingredients as shown in Table 3.1.1. They are produced from biomass through fermentation or enzymatic hydrolysis.

Table 3.1.1 Common bio-based food and feed ingredients derived from food industry (Tsagaraki et al., 2017)

Food industry sector	Relevant bio-based products
Fruit and vegetable	Glucose, citric and linoleic acids, tocopherols, polyphenols, ascorbic acid, dietary fiber, pectins, and pigments.
Grain processing	Vitamins, minerals, key unsaturated fats, β-glucans, dietary fibre, antioxidants and different sterols.

Brewery and winery	Flavouring agents, enzymes, single cell protein (SCP), substrate for microalgae cultivation, cellulose and non- cellulosic polysaccharides, sugars, proteins, acids and antioxidants.
Marine	High caliber protein, lipids with high levels of unsaturated fatty acids, hydrolysates from fish guts/cleanings, peptides, and products from crustaceans such as chitosan, chitosan oligomers, glucosamines, omega-3 oils, chitin and chitosan, fish protein hydrolysates, algal constituents, carotenoids and antioxidants.
Meat	Bioactive peptides using hydrolysis or fermentation with antimicrobial, antioxidative, antithrombotic, antihyper- tensive or anticancerogenic activities.
Dairy	Solvent proteins (β-lactoglobulin, α-lactalbumin, immunoglobulin, bovine serum albumin, lactoferrin, and lactoperoxidase), high content of essential amino acids with antimicrobial, anticarcinogenic or biological activities.

Bio-based food and feed ingredients constitute a growing market with big demand and competition. Bio-based food and feed ingredients have numerous applications in various sectors of the food industry such as dairy products, beverages, oils and fats, animal feed, infant nutrition, snack foods and bakery goods etc. and fall in six major categories:

- ✓ ***Probiotics***: This term is used to name edible microorganisms with several associated health benefits for humans and animals. The probiotics market in Europe was valued at 100 million EU in 2010. The Western European market for probiotic bacteria cultures is focused on four areas – dairy products, animal feed, supplements and infant nutrition – and it was valued at EUR 30-36 million, and the US market was worth EUR 127 million;
- ✓ ***Prebiotics***: Fructo-oligosaccharides that, when digested, have a positive impact on the composition of bacteria in the digestive tract. Nearly all carbohydrate-based food products, such as bread and cereals, but also margarines, fruit juices and dairy drinks, can be enriched with prebiotics. The fructan market (which includes inulin and fructo-oligosaccharide) is the biggest segment. The fructan market reached EUR 180 million in 2010, attracting several new market participants;
- ✓ ***Dietary fibers***: Dietary fibers are increasingly used to replace fat in food dietary products;
- ✓ ***Peptides***: Bioactive peptides and proteins are a group of functional ingredients of growing interest due to their functional properties, e.g. anti-cholesterol, anti- inflammatory and antioxidant properties. Their major applications are in sport drinks and animal feed;
- ✓ ***Terpenes***: The terpenes sub-category of carotenoids includes beta-carotene, lycopene, astaxanthin and lutein – which are also widely used for animal feed, as food coloring, in cosmetics, in pharmaceuticals and in dietary supplements. The beta-carotene on the market is usually synthetic and its production is concentrated at DSM and BASF, which have a combined share of over 80%. However, the use of naturally- derived carotenoids is increasing, particularly in functional food and health food applications;

- ✓ ***Phenols***: Polyphenols, being traditional food colorants, are increasingly marketed as antioxidant food ingredients. Latest researches focused on the study of polyphenols suggest that these molecules can protect against cancer and cardiovascular diseases, as well as increase anti-inflammatory activity.
- ✓ ***Nutraceuticals***: The term "nutraceutical" is a blend of two words: "nutrient" (a nourishing food compound) and "pharmaceutical" (a medicinal product). The term was invented in 1989 by Stephen DeFelice, founder and chairman of the American organisation named Foundation for Innovation in Medicine. Nutraceuticals is a broad term that is used to describe any product obtained from food sources with more health benefits in addition to the basic nutritional value found in foods. They can be considered non-specific biological therapies used to stimulate general well-being, control symptoms and prevent malignant processes.

Bio-based animal feed co-products are also generated through biorefinery processing for the production of biofuels, mainly bioethanol and biodiesel (Table 3.1.2). Currently, co-products are an important feed resource in many countries, for ruminants, non- ruminants and aquaculture. Due to the expansion of the feedstock used for bio-fuels production (from first to second generation), several co-products have been introduced in the global market and can be used as livestock feed. This trend is on-going, increasing both in complexity and in the number of livestock species that are benefiting.

Table 3.1.2 Feedstock used for bioethanol and biodiesel production, their feed co-products and major areas of utilisation (Tsagaraki et al., 2017)

Feedstock	Co-product	Co-product utilisation for animal feed
Maize **Sorghum** **Wheat** **Triticale** **Rye** **Barley** **Co-products from biodiesel production**	Distillers grain (DG) Wet distillers' grain (WDG) Dried distillers' grain (DDG) Dried distillers' grain with added solubles (DDGS) DDGS with high protein additive Maize oil, maize-condensed distillers solubles, maize gluten feed	Distillers' grains (DG) Distillers dried grains with solubles (DDGS) Wet distillers' grain (WDG), DDGS-HP for beef cattle DG for dairy cattle DG for pigs DG for poultry DDGS as grazing supplements for ruminants DDGS for aquaculture
Sugar cane **Sugar beet** **Sweet sorghum** **Cassava**	Vinasse (multi-nutritional blocks/ pellets/meal) Sugar cane tops, bagasse and molasses Sugar beet tops, fermentable palatable waste Grain bagasse/foam/froth/steam/vinasse/ syrup from 'sugary stems' Cassava residue plus sludge from cane processing	Sugar cane co-products including use of effluents for cattle Sugar cane bagasse with supplements and cassava residue for cattle and other ruminants
Soybean **Rapeseed** **Vegetable oils** **Maize oil**	Crude glycerine Oil seed cake (mechanically extracted) and meal (solvent extracted) after methanol removal	Oil seed cake and meal used for pigs, beef cattle, fish, dairy, poultry Glycerol as drench and supplement for dairy cattle
Camelina sativa	Camelina meal (no irrigation needed, rich in aminoacids and antioxidants)	Poultry (broilers and layers)

Jatropha	*Jatropha platyphylla* and *Jatropha curcas* Kernel meal and protein isolate (heated, detoxified)	Fish, turkeys and pigs
Oil palm Babussa	Oil palm fronds, trunks, pressed fibre, empty fruit bunches, kernel cake, oil mill effluent	Ruminant feeding and complete diets based on oil palm for poultry, pigs and freshwater fish
Seed oils	Co-products derived from bioethanol and biodiesel production	In livestock feed as feed additives

3.1.3 Bio-based products production processes

The processes used to produce bio-based products from biomass belong to four main groups (Table 3.1.3):

- ✓ ***Mechanical or physical processes*** (milling, separation, upgrading, dehydration etc.) are mainly used as biomass pre-treatments or in intermediate processing stages;
- ✓ ***Chemical processes*** (hydrolysis, oxidation etc.) are also mainly used as biomass pre-treatments or in intermediate processing stages;
- ✓ ***Biochemical processes*** (anaerobic digestion, fermentation, transesterification etc.). Fermentation processes have been used since World War I for industrial production of energy carriers and chemicals. Many bio-based chemicals are now manufactured by fermentation at an industrial scale, such as ethanol, lactate, amino acids and citric acid with the potential to produce many more. Apart from sugar and starch feedstock, lignocellulosic feedstock is also tested in more advanced fermentation systems. Anaerobic digestion is a well-established process for biogas production
- ✓ ***Thermochemical processes*** (combustion, gasification, pyrolysis etc.) are suitable for the conversion of lignocellulosic feedstock, such as wood. Incineration is the most widely used process to produce bioenergy for heat or power. Gasification technologies are under development and few large commercial units exist, mainly in areas with substantial forestry biomass capacity. Pyrolysis technologies are not yet fully commercially mature with a number of pilot plants in use worldwide and they can be economically efficient for Combined Heat and Power (CHP) only or in a biorefinery concept where both energy and other products of higher value are produced. Liquefaction and torrefaction of biomass are not well developed and are expensive, although they have several potential applications.

Table 3.1.3 Bio-based products and related processes (Tsagaraki et al., 2017)

Mechanical/ Physical processes	Biochemical processes	Chemical processes	Thermochemical processes
Pressing	Anaerobic digestion	Hydrolysis	Combustion
Milling	Aerobic/Anaerobic fermentation	Oxidation	Gasification
Separation	Enzymatic conversion	Pulping	Pyrolysis
Fiber separation	Transesterification		Hydrothermal upgrading
Upgrading			Torrefaction
Fractionation			Liquefaction
Extraction			Hydrogenation

3.1.4 Biowaste Valorisation

Bioeconomy proposes an opportunity to transform the bio-waste from a cost into a resource. Waste biomass offers a potential way to overcome the concerns over using food materials for nonfood purposes regarding bio-based production and there is growing acknowledgment of the benefits of using wastes and residues as feedstock.

Used cooking oils (UCO) or recycled vegetable oils were the second most important feedstock for biodiesel production in 2015 in the EU, with the Netherlands, the UK and Germany being the largest EU producers of biodiesel manufactured from used cooking oils. It has attracted a considerable interest for aviation biofuels production. Industry analysts estimate that more than million tons of UCO was consumed in Europe in 2013. Of this volume, about 700.000 tons are estimated to come from within the EU, as there are substantial imports of used cooking oils, largely coming from the US. Other oils originating from plants include tall oil, residue from pulp industry, palm fatty acid distillate and by-products of the production of Omega-3-fatty acids from fish oil. Finally, waste from potato processing industries (potato peel) has drawn interest in being used as a bioethanol feedstock in recent years.

Waste animal fat is also used for biodiesel production in multi-feedstock production facilities of small and medium-scale, referred to as Animal Fatty Acids Methyl Esters (FAME), which displays better engine performance than conventional biodiesel according to recent surveys.

Municipal Solid Waste (MSW) can also be converted into liquid biofuels or used for heat and power generation. European households dispose of around 110-150 million tons of biogenic material annually. The different MSW streams are:

- ✓ ***Recyclable materials*** (metals, paper and plastics): used for manufacture of recycled products;
- ✓ ***Organic fraction*** (putrescible food waste, garden waste): may be converted to biogas via anaerobic digestion. It is estimated that around 44 million tons of household and garden waste will be available in 2030. Estimations of food waste quantities are extremely difficult. The European Commission estimated a total of 89 million tons of food waste produced in 2010 in the EU, including manufacturing food waste (35 million tons), household food waste (38 million tons), retail/ wholesale food waste (4 million tons) and food service/ catering food waste (12 million tons);
- ✓ ***Solid Recovered Fuel*** (SRF) (the fraction of MSW that cannot be recycled, e.g. shredded textiles, wood, paper, card and plastics): SRF can be combusted or converted to syngas, and then be used for bioenergy or be processed into advanced biofuels. Estimates of the wood fraction of MSW are in the range of 26-57 million tons per year. Of this, around 40 % is recycled into other products and around 50 % is burned for energy, leaving just under 10 % available as potential feedstock for advanced biofuels.

Food waste is a part of the organic fraction. It is not suitable for direct energy generation through conventional burning processes because it has high moisture content. Biological technologies such as anaerobic digestion (AD) are more suitable, and there is the technical possibility of co-digestion with other substrates such as manure and slurry. But even in this case the processing is

expensive and technically complex due to the heterogeneity of food waste derived from diffuse sources and the need of separation/ pretreatment. The diffuse sources of food waste and the fact that in most cases food waste is blended with other types of waste from households constitute a great challenge with respect to collecting, transporting and separating this waste stream, as well as to locating possible processing plants. Source separation and collection of food waste would provide the greatest potential for its use. Finally, a further potential barrier to the mobilisation of food waste as a source of energy is the fact that increasing legislation and policy efforts are being made in the EU to prevent and reduce food waste. This may limit possible investments in relevant process infrastructure and technologies.

Finally, any organic residues / biological waste materials can potentially be converted to advanced biofuels by thermochemical, biochemical or chemical processes. Examples of such biomass feedstock include waste from beer and other beverage production and waste from bakeries etc. The facilities that use this kind of feedstock follow a biorefinery approach improving the conversion of biomass and waste streams into products and energy.

3.1.5 References

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Course 3.2: Novel bioproducts development – strategies

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3.2.1 Introduction

The increasing human population, the expanding energy costs, the use of fossil fuels, and the concerns about the global climate change are mainstreaming the worldwide efforts towards transition to a sustainable, 'green' bio-based economy. The transition is a complex process; it will require huge biomass feed-stocks and versatile technologies for their processing, operating in an eco-friendly manner to produce novel bio-based products.

The sustainability principles of economic development are grounded on strategies that outline the main pillars of the global policy towards a climate-neutral society by 2050. The sustainable fuels, energy, and products are in the focus of the international research and industrial development. There are regular policy updates on this development. However, Energy & Environment policies from a research and innovation perspective are among the leaders in the agenda.

The European Energy Research Alliance (EERA, https://www.eera-set.eu/) is the largest energy research community in Europe. It is a membership-based, non-profit association and brings together 250 universities and public research centres in 30 countries. EERA's joint research programmes cover the whole range of low-carbon technologies as well as systemic and cross-cutting topics. EERA has launched a Joint Programme on Bioenergy (JP, http://www.etipbioenergy.eu/supporting-initiatives-and-platforms/ec-rd-d-networks-and-initiatives/european-energy-research-alliance-bioenergy-joint-programme-eera-bioenergy-jp) and corresponding Strategic Research and Innovation Agenda (SRIA) The overall objective of the EERA Bioenergy Joint Programme is to align pre-competitive research activities at EERA institutes to give a technical-scientific basis to further develop the next generation biofuels routes and to explore the possibilities for joint technology development. The more efficient use of R&D investments that this Joint Programme foresees, contributes to an acceleration of the development of next-generation conversion technologies.

SRIA is developed to satisfy the main aims of the JP, as follows:

- ✓ Line up research activities to form the scientific and technical basis to further development of advanced bioenergy routes and to promote the possibilities for joint technology development.
- ✓ Line up research priorities and activities with other external stakeholders, while encouraging international co-operation as well.
- ✓ Evaluate research and innovation priorities to accelerate the implementation of the bioenergy sector in Europe.
- ✓ Be active in the research, development, and innovation.

SRIA addresses, among others, the decarbonisation of the energy sector. An essential component of this sector is the sustainable production and processing of biomass, the bioenergy gaining and use, the production of bioproducts, and their public acceptance.

Thus, shaping the strategies for novel bioproducts development, the following important considerations are taken into account.

- ✓ **The development of sustainable biomass feedstock**. Availability of sufficient quantities of biomass feedstock is the bottleneck of both bioenergy and bioproducts production. Here, the potential of biomass resources that are either underused or simply unused must be explored. This is an approach that can be considered to satisfy the constantly increasing biomass demand.
- ✓ **Assurance of the sustainability of the systems for production of bioenergy and bioproducts**. The biomass demand must be considered in the context of biomass value chain, and consequently – the efficiency of the technologies for its conversion in respect to costs, carbon balance, and quality of the feedstock, must be regarded. The biomass value chain (BVC), also referred to as biomass supply chain (BSC), is characterised by the valorisation of biomass feedstock for the production of bioenergy and innovative bio-based products (https://www.sciencedirect.com/science/article/abs/pii/S0960308519300641)
- ✓ **The essential role of innovation**. The technologies have to meet the highest levels of efficiency and low carbon use while reducing the costs of bioproducts production. This approach is an essential part of the research area of the Strategic Research and Innovation Agenda.
- ✓ **Process and system integration**. The approaches for integration of the processes and the systems possess great opportunities to improve efficiency and decrease the costs of bioproducts production. Through these approaches, higher integration level of the activities of the JP will be achieved.
- ✓ **Increasing the role of Renewable Energy Sources (RES)**. Renewable Energy Sources are those renewable resources, which are naturally replaced on a human timescale and are used for obtaining energy. Examples for such resources include sunlight, wind, rain, waves, currents, and geothermal heat (https://en.wikipedia.org/wiki/Renewable_energy). The research on the synergies of bioenergy with other discontinuous RES aims to increase the efficiency and quality of the energy provided to make the production of renewable fuels.
- ✓ **Sustainability and economic competitiveness**. These are the two basic issues for achieving social acceptance of bioenergy and bioproducts.
- ✓ **Alignment and cooperation with research institutions and industries**. These are also key aspects for successfully implementing the SRIA.

3.2.2 Sustainable production of biomass

The research focus on sustainable biomass production is placed on making best use of biomass resources for conversion plants while assuring safety and flexibility of supply, quality of the biomass, ecological sustainability, and decrease of the costs of biomass feedstock. Biomass feedstock is an alternative to finite fossil fuel resources, since it offers most of the benefits for energy and products applications compared to the former (https://www.sciencedirect.com/topics/chemistry/biomass-feedstock).

This research is expected to significantly contribute to increase in conversion efficiency, along with a synchronised reduction in production costs for advanced bio-products by 2030, compared to the present situation.

There are four kinds of biomass: **forest biomass, agricultural biomass, algae biomass, and biogenic waste biomass**, that need practical advance in public acceptance and in the security of a long-term sustainable supply to biomass conversion plants.

The biomass research priorities concern the following.

Forest biomass:

- Increasing forest resilience towards climatic accidents.
- Optimising business concepts for forest harvesting operations.
- Development of new management practices with low environmental impacts in line with the development of multicriteria assessment tools and methods for forest biomass.
- Development of models to be used as decision support tools to organise the forest biomass market.

Agricultural biomass feedstock: The sustainable use of agricultural resources demands efforts to increase the economic competitiveness of producing biomass while reducing the environmental impact. In this context, knowledge about the various types of biomass is required, and the research activities must be focused on:

- Increasing the knowledge of agricultural biomass utilisation by defining appropriate agricultural management solutions to reduce the environmental impact.
- Intensifying agricultural production through design and optimisation of innovative systems for agroforestry.
- Optimisation of feedstock supply systems and logistics chains through yield improvements, environmental impact reduction, and exploration of new biomass resources.
- Evaluation of the impacts of biomass agricultural production systems on the environment and the certification schemes and public policy frameworks through Life Cycle Analysis (LCA) of whole value chains.

***Biomass from microalgae and seaweed (macroalgae*)**: Algal biomass possesses important potential advantages but also some constraints. Potential advantages of algal biomass are:

- ✓ Much higher specific energy production surface compared to terrestrial crops;
- ✓ Absence of polymers such us lignin, which facilitates the conversion processes;
- ✓ Possibility to be cultivated in artificial installations under controlled conditions (especially microalgae), which may have positive effects on maximising biomass yield and allows the use of industrial CO2 flows.
- ✓ Large variety of molecules that can be extracted as valuable food and non-food bio-based products, as well as mineral content that can be used to close the mineral fertiliser cycle.

However, the competitive production and use of algal biomass imposes significant constraints, such as:

- ✓ The scaling-up of production technologies;
- ✓ Very high production costs;
- ✓ Rates far below EROEI (energy returned/energy invested).

The research priorities for improving the energy efficiency, the environmental sustainability, and the economic competitiveness of algal biomass to produce bio-products are focused in the following directions:

- Genetics: selection and genetic manipulations of algal species/strains for improvement of physiological and technological characteristics to achieve enhanced overall efficiency of the production process, biomass yield and its harvesting, extraction and productivity of target final products, and restriction of contaminationation. All these characteristics can have a positive impact on the production process.
- Techniques/Technologies: technical/technological innovations in microalgae cultivation and harvesting processes for diminishing energy demands and costs for lightening and harvesting and optimising of the lightening systems operation in the photobioreactors. Regarding macroalgae, the research trends encompass development of automated systems for harvesting and effective techniques for fast stabilisation and long-term storage prior to use.
- Mathematical modeling. Creation of predictive models for optimal cultivation and harvesting of macroalgae, respecting the indicators seaweed quality and most appropriate harvesting time.
- Systems integration. Coupling of biofuel production with the extraction and marketing of valuable products, such as proteins, antioxidants, pigments, etc.

***Biogenic waste*:** Biogenic waste includes urban, agro-industrial, and green waste; livestock waste, and other sources of organic material. It can be transformed through thermo- and bio-chemical, conversion or via anaerobic degradation depending on the water content of the raw material (low, for the former or high for the latter types of bio-waste). The conversions result in obtaining advanced biofuels and bio-products – soluble materials rich in organic matter, which have diverse practical applications: from heating to electricity production, to bio-hydrogen and bio-methane.

The biogenic waste management and application require strategies grounded on research along the entire waste value chain. The stages considered are: mobilisation of the waste feedstock, processes of its transformation and the recovery, and application of the obtained end products.

The research studies need to be organised in a way that achieves two mayor objectives:

1. implementation of safety measures while manipulating the waste to prevent exposure to health risks;
2. minding the procedures of the conversion technologies to increase the transformation yield.

Research priorities regarding the waste value chain stages cover:

- Waste feedstock mobilisation: There is a gap in choosing the best methods to collect and store waste feedstock in order to guarantee its regular and

secure supply. In this context, research efforts must be focussed on development of technologies for eliminating contaminated pathogenic microorganisms, for efficient pre-treatment procedures, and for removal of the unusable fractions in the waste feedstock.

- Development of better recovery technologies that consider important issues, such as:
    - Integration of the conversion process;
    - Extended uses of waste feedstock for production of bio-based products;
    - Expoiting digital predictive models for development of efficient anaerobic digestion technologies using criteria such as the waste quality, the environmental impact, especially the effect of the waste-derived biofertilisers on soil;
    - Public acceptance of waste technologies in terms of social and economic risk analyses.

3.2.3 Thermochemical processing of biomass

The main research strategies regarding thermochemical processing of biomass are concentrated on rising the sustainability of the process. These include measures to increase the efficiency, to reduce the GHG emissions, and to realise a cost-competitive production of advanced biofuels and bio-products.

The identified research areas/objectives are related to:

- Development of primary thermochemical conversion processes;
- Downstream processing;
- Advanced biofuel and intermediate carrier value chains.

For these objectives to be reached, KPIs are determined with a prospect levels of 2030 compared to 2020 ones. In the context of these KPIs and the challenges that they impose, the underlying principles in the research strategies are specified. They include:

- Simplification and integration of the thermochemical conversion process in order to increase its reliability;
- Increase the feedstock repertoire: use of waste or high- and low-grade biomasses, and new biomass sources (e.g. algal biomass);
- Co-use of biomass processing products with other sources or use of bio-based products for obtaining biofuels;
- Create negative GHG emissions by combining bioenergy with carbon capture.

✓ **Development of primary thermochemical conversion processes**

The primary thermochemical biomass conversion processes are gasification, torrefaction, hydrothermal processing, and pyrolysis.

Biomass gasification results into gaseous intermediates: syngas and product gas. The former is the raw material to synthesise different chemicals and bio-products. The processes of biomass gasification have not yet been commercially implemented because they need substantial reduction of the costs and

improvement of their reliability. That is why, relevant research topics are recognised to be implemented in the long-term research strategies:

- Utilisation of low-cost materials like bio-wastes, by-products of biochemically processed biomass;
- Improvement of gasification performance in terms of feedstock and technical/technological characteristics;
- Optimisation of product gas composition for downstream processing;
- Innovation of gasification processes.

Biomass pyrolysis is defined as fast and slow, and while the former yields liquid bio-oils, the latter produces charcoal. The pyrolytic oil can be either upgraded or directly used as heating oil.

To improve the process performance (e.g. to reduce costs, improve the efficiency of the production process and the quality of the process products), several key research topics are addressed:

- Standardisation of the properties of bioenergy carriers;
- Increasing feedstock repertoire;
- Development of new models to better understand the pirolytic processes.

Torrefaction technology concepts have been already put into practice; the first full-scale commercial plants are built. However, their commercial implementation needs both research and technical work in order to make this type of thermochemical biomass conversion process more competitive and in line with new bioproducts applications.

The relevant research topics that will lead to better future for these technologies include:

- Improving the quality of the torrefied pellets (the final product);
- Development of safety protocols and standards for the product quality while lowering the energy and investment costs;
- Developing new, high added value products.

Hydrothermal processing technologies are still under laboratory and pilot studies. These technologies involve water treatment thermochemical processes that yield a variety of products: solid (biochar), liquid (bio-crudes), and gaseous (methane, hydrogen, etc.), and other chemicals. The scale-up of these processes is a complicated issue due to the existing process and technological barriers, the main of which are.

- Still high production costs;
- Significant negative environmental impact;
- Lack of advancement in the process performance and the equipment design.

That is why, the research efforts are focused on topics like:

- Expand the understanding of the basic mechanics of the process, incl. reaction kinetics;
- Use of catalysts;
- Development of common and standardised analytical methods.

- ✓ **Downstream processing**

The processing of downstream products needs optimisation is terms of cleaning, conditioning, and upgrading.

Downstream processing optimisation

The optimisation of gas cleaning processes requires approaches that:

- Combine increasing efficiency while reducing the processing costs;
- Improve sample measurement and control techniques;

In addition to the cleaning procedures, clean gas conditioning and up-grading are also essential and they are readily performed through catalytic processing and separation technology application. The tuning technologies for conditioning clean biomass gas include the development of improved catalysts, sorbent and/or membrane formulations that are more tolerant to biomass-derived contaminants, along with efficient procedures for regeneration and recycling. These are key research topics to improve the processes flexibility and competitiveness.

Further research input is needed as well for conditioning and upgrading of biocrude fraction resulting from pyrolysis and hydrothermal processing of biomass. Biocrude conditioning mostly involves removing ash to avoid undesired polymerisation of the components. Here, the efforts must be concentrated on:

- Development of conditioning processes for better storage and use of the products;
- Upgrading the conditioned biocrude by catalytic hydrogenation at high pressure;
- Developing catalysts that are optimised for that process;
- Development of treatments and uses for aqueous effluents, including by-product recovery that converts the organic compounds contained in the effluents into combustible gases.

- ✓ **Advanced biofuel and intermediate carrier value chains**

To realise successful conversion of biomass into advanced biofuels and intermediate bioenergy carriers with high GHG savings, the individual unit operations must be unified into a smart biomass-to-by-products value chain design.

Smart biomass-to-by-products value chain

To realise such industrial symbiosis, process simplification and integration is needed to improve the performance of the processes. In other words, the thermochemical and biochemical based processing must be combined along with as high as possible internal recycling of the waste streams. On the other hand, considering the end products, smart schemes that accentuate on the combined production of energy and bio-based products, while utilising the extra H_2 and CO_2 flows, are needed. They will certainly contribute to the overall sustainability of the processes. Finally, testing and demonstration of these options are needed for their technical assessments. Hence, relevant research, development and innovation activities must be undertaken. The strategic priorities in this context are:

- Development of innovative chain design: optimising the production systems; use of main syngas components to produce higher value bio-based products, use of charcoal as soil fertiliser; developing integrated

gasification-based biofuel production with renewable hydrogen to reduce the GHG emmissions;

- Development of options for co-production of biofuels with chemicals, and optimising integration aspects;
- Integrating the overall bioenergy carrier production process with other industrial processing with surplus heat;
- Development of smart system designs with integrated non-energy co-products. Here, the recovery of plant nutrients from the aqueous phase, integrated hydrogen recovery for upgrading processes, conversion of aqueous phase compounds into chemicals, etc. are considered, as well as the system design optimisation through integration.

✓ **Biochemical processing of biomass into advanced bio-based products**

The scope of this research strategy is to implement the biochemical and chemical processes and technologies for co-production of advanced biofuels other bio-based products in biorefinery approaches, including the biogas from anaerobic digestion, the syngas from thermochemical biomass and bio-waste processing, and the hydrogen from biological and renewable origin.

Alongside the entire conversion schemes, from the biomass pre-treatment to the recovery of side-streams and integration of bioprocessing technologies, needs have emerged for technological innovations and novel concepts development in the field of (bio)catalysis. New or improved process catalysts are desired to enhance the biological efficiency and product yields from the conversion process. The development of such catalysts is emphasised as a major research, development and innovation challenge.

The KPIs defined in the corresponding research strategy are an increase in the net efficiency of biomass conversion and a significant reduction in the production costs. To satisfy the KPIs, the main research areas are identified as:

- Development of enzymes and cell factories;
- Increasing the efficiency of microbial and algal biochemical pathways;
- Design of novel pathways and microorganisms to biochemically convert biomass into advanced biofuels and bio-based products.

The enzymes and cell factories research area involves improving the robustness and efficacy of the total enzymes mixture used in biochemical processing of biomass while reducing the costs, and respectively – the cost of the technologies. Additionally, the design of novel enzyme possessing improved catalytic activity of broader substrate spectrum is also considered. The approaches for development of new/optimised enzymes foresee obtaining biocatalysts with increased efficiency and reduced production costs. This improvement will reflect the whole conversion process, since the enzymes are its main component regarding the technology economics.

Increasing the efficiency of microbial and algal biochemical pathways. Here, metabolic engineering strategies are concerned with the scope to deregulate the metabolism of the microbial cells in a way that the negative influence on the conversion processes is minimised. These strategies encompass tuning appropriate metabolic chains to increase the efficiency of the natural biochemical

pathways in microbial and algal cell factories. Among others, the following research topics are identified:

- Construction of microbial strains with uncoupled growth and fermentation to achieve maximal fermentation activity in non-growing cells;
- Construction of microbial strains with metabolic rearrangements that result in increased yield of the desired final products (e.g. through increasing the activity of auxiliary metabolic pathways);
- Genetic engineering of microbial strains with improved characteristics for syngas conversion;
- Induction of mixed cultures or engineered microbial strains to express a larger number of the enzymes involved in the conversion processes. Examples in this context can be genetically engineered bacterial strains that express a coctail of hydrolytic enzymes involved in fermenting lignocellulose or non-conventional yeasts with increased carbon conversion efficiency in the production of long-chain fatty acids.

Design of novel pathways and microorganisms. This is an ambitious goal whose strategic recourse reaches beyond 2030 and envisages construction of artificial cell factories.

In addition to these research areas, other strategic research priorities include:

- Improvement of the current technologies and developing new ones for feedstock preparation, deconstruction and fractionation on the basis of flexible pretreatment methods.
- Development of solid materials for syngas and biogas cleaning and up-grading, such as catalytic membranes, zeolites or novel solid absorbents.
- Improvement of the current methods and developing new ones for algae fractionation through a cascade approach. This approach considers not only the efficient separation of the biomass processing fractions but also the preservation of the characteristics of the high-value, bio-based products thus obtained. Examples of such novel product-friendly technologies are supercritical fluids, ultrasound and microwave assisted extractions, and pressurised extraction.
- Improvement of the pre-treatment stage. Here, the efficiency of bio-processing for ethanol, higher alcohols, fatty acids, hydrocarbons, and hydrogen are regarded. The research topics on this issue are the development of more robust production strains (yeasts and bacteria), with higher resistance to inhibitory compounds present in the cell factories, and with the capacity to transform inhibitory fermentation products into bio-based products (e.g. engineered *Clostridium* sp. strains transforming butanol into non-toxic ethers or esters).
- Improvement of the efficiency of (bio)catalytic upgrading of intermediate bioprocessing products into advanced bio-based products. For instance, the development of novel solid materials for direct catalytic upgrading of biomass hydrolysates to produce bio-based products from alcohols contained in fermentation broths.
- Improvement of carbon conversion efficiency to achieve the cost-competitive conversion of gas flows from biomass thermochemical and biological processing into advanced biofuels and bio-based products.

Increasing the cost competitiveness of using algae and bacteria to produce bio-hydrogen and biomethane is also a key issue for the long-term future production of advanced biofuels and bio-based products.

Process, mass and energy integration coupled to waste and byproduct integration is the overall goal of any conversion technology focused on minimising GHG emissions and aiming to reach zero effluents. These challenges are research priority, addressed by:

- Development of *in situ* product recovery (ISPR) technologies that result in increased product yields and reduced economic and energy costs.
- Life Cycle Analysis of the value chains of the bio-based products obtained in the biochemical-based biorefineries: from feedstock to final product use and disposal or recycling. This is a well-recognised tool to determine the economical, environmental, and social dimensions of the conversion prcess.
- Recovery of process side-streams into biofuels and other bio-based products. The aqueous fraction resulting from the fractionation of bio-oils, which contains a diversity of organic oxygenated compounds, can be used as a source of hydrocarbons and aromatics obtained through the application of newly-designed solid catalyst that allows this transformation.

3.2.4 Sustainability/ Techno-economic analysis/ Public acceptance

Regarding sustainability, a thorough understanding of not only environmental-related aspects, but also the broader sustainability dimensions of bioproducts development, such as economic and social is needed. Thus, the following four focus analysis areas can be defined: environmental, techno-economic, social, and cross-cutting sustainability.

- Environmental Analysis. The research strategies for the environmental analysis encompass comprehensive assessment of the environmental implications of bioproducts development, and understanding their potential for achieving environmental goals. Here, the climate-change related impacts of bio-based production technologies in meeting the climate mitigation challenge are considered. Issues related to methods for assessment of the environmental impacts are also considered.
- Techno-Economic Analysis (TEA). Conceptual Design and TEA of biorefineries and biomass conversion processes and Metrics for assessing the economic sustainability of the processes are the two major research trends. Topics, such as profitability analysis and development of supply chain models and optimisation of economic performance along the production chain are considered.
- Social Analysis. The public's perception/acceptance of bio-based products development and application, including which factors, strategies or policies may play a role is an important social aspect that needs better understanding.

- Cross-Cutting Sustainability Analysis. An overall sustainability assessment should cover the economic, social and environmental pillars and identify potential synergies/compromises between these dimensions. The cross-cutting sustainability analysis as a research strategy that addresses the bio-based products development through questions such as socio-economic impacts, links with the UN's Sustainable Development Goals (SDG's), role in the Circular Economy. Finally, the political and regulatory framework for bio-based products development in Europe is also considered.

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Course 3.3: Bioproducts (incl. fisheries and aquacultures) validation in an operational environment

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3.3.1 Introduction

Bioproducts (including those of fisheries and aquacultures) for human or veterinary use are subject to validation in an operational environment in order to ensure safety and quality standardization. This is outlined in detail in a series of guidelines; most notably in several relevant Codex Alimentarius documents and EMEA's *Note for guidance on specifications: Test procedures and acceptance criteria for biotechnological/biological products* (CPMP/ICH/365/96). The procedures apply to the stages of development and processing, the employment of reference standards and validated analytical procedures, all stages of process control (from development to commercialization) and quality characterization (specifications).

Codex Alimentarius (i.e. *food code,*) adopted by the Codex Alimentarius Commission (CAC), which is the core of the Joint FAO/WHO Food Standards Programme, is a collection of internationally adopted food standards, guidelines and codes of practice intended to contribute to the safety, quality and fairness of international food trade by facilitating the harmonization of definitions, requirements and practices, and consequently, international trade. Noteworthy, Codex standards and related texts are not a substitute for, or alternative to national legislation. In addition, Codex standards are subject to revision based on advancements in knowledge. The Codex Alimentarius includes standards for all the principal foods, whether processed, semi-processed or raw, for distribution to the consumer. Materials for further processing into foods should be included to the extent necessary to achieve the purposes of the Codex Alimentarius as defined. The Codex Alimentarius includes provisions in respect of food hygiene, food additives, residues of pesticides and veterinary drugs, contaminants, labelling and presentation, methods of analysis and sampling, and import and export inspection and certification.

This Unit mainly follows EMEA's guidelines, with additional reference to related Codex Alimentarius texts. More details for foods derived from biotechnology see in Codex Alimentarius guidelines. Some Codex documents relevant to biotechnology products and foods derived from fisheries/aquaculture are listed in Table 3.3.1 . Bioroducts should cause no toxic, allergenic or mutagenic effects. In the case of fish side streams used for human grade food, the quality and safety of the raw materials have to be ensured by handling and controlling side streams in the same manner as the primary product. That is why food safety and quality control systems are applied, including Hazard Analysis and Critical Control Point (HACCP) and Good Manufacturing Practice (GMP). There should be appropriate control on all biological and chemical hazards. To develop appropriate food safety controls, a proper understanding of the association between reduction in hazards associated with food and reduction in risk to consumers is of central importance. To protect the ecosystem and public health, indiscriminate and unregulated use of chemicals and therapeutics in aquaculture should be discouraged **(5)**. The

legal framework on food additives in EU is defined in Regulation (EC) Nº 1333/2008.

Table 3.3.1 Codex Alimentarius documents relevant to foods derived from modern biotechnology and/or fisheries and aquacultures. For more information on these and other Codex documents, visit http://www.fao.org/fao-who-codexalimentarius/codex-texts/guidelines/en/ .

Ref. no.	Document	Committee	Year
CXG 44-2003	Principles for the Risk Analysis of Foods Derived from Modern Biotechnology	TFFBT	2011
CXG 76-2011	Compilation of Codex texts relevant to the labelling of foods derived from modern biotechnology	CCFL	2011
CXG 68-2008	Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Animals	TFFBT	2008
CXG 74-2010	Guidelines on Performance Criteria and Validation of Methods for Detection, Identification and Quantification of Specific DNA Sequences and Specific Proteins in Foods	CCMAS	2010
CXG 48-2004	Model Certificate for Fish and Fishery Products	CCFFP	2004

Note: TFFBT, Ad Hoc Intergovernmental Task Force on Food Derived from Biotechnology; CCFL, Codex Committee on Food Labelling; CCMAS, Codex Committee on Methods of Analysis and Sampling; CCFFP, Codex Committee on Fish and Fishery Products

3.3.2 Extensive bioproduct characterization at development and processing stages

Bioproducts obtained from fisheries and aquacultures, like any biotechnological products, must be extensively characterized at development and processing stages to define strict specifications in terms of physicochemical properties, biological activity, immunochemical properties, purity, impurities and contaminants.

- ✓ ***A specification*** is defined as a list of tests, references to analytical procedures, and appropriate acceptance criteria which are numerical limits, ranges, or other criteria for the tests described. It establishes the set of criteria to which a drug substance, drug product or materials at other stages of its manufacture should conform to be considered acceptable for its intended use. "Conformance to specification" means that the drug substance and drug product, when tested according to the listed analytical procedures, will meet the acceptance criteria. Specifications are critical quality standards that are proposed and justified by the manufacturer and approved by regulatory authorities as conditions of approval (EMEA's guidelines https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-6-b-

test-procedures-acceptance-criteria-biotechnological/biological-products-step-5_en.pdf).

- ✓ ***Biological activity*** is the specific ability or capacity of the product to achieve a defined biological effect. Potency is the quantitative measure of the biological activity (EMEA's guidelines https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-6-b-test-procedures-acceptance-criteria-biotechnological/biological-products-step-5_en.pdf).
- ✓ ***Impurity*** is any component present in the drug substance or drug product which is not the desired product, a product-related substance, or excipient including buffer components. It may be either process- or product-related. (EMEA's guidelines https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-6-b-test-procedures-acceptance-criteria-biotechnological/biological-products-step-5_en.pdf).
- ✓ ***Contaminants*** are any adventitiously introduced materials (e.g., chemical, biochemical, or microbial species) not intended to be part of the manufacturing process of the drug substance or drug product (EMEA's guidelines https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-6-b-test-procedures-acceptance-criteria-biotechnological/biological-products-step-5_en.pdf).

Bioproducts need to meet certain predefined acceptance criteria. The main aspects that need verification concern the consistency of manufacture and the stability and safety of the bioproduct.

For example, in the case of medicinal products for human use (including biopharmaceuticals), EMEA's guidelines require extensive characterization to be done in the development phase as well as following considerable process modifications. Ideally, this characterization will include comparison with a reference standard (refers to international or national standards) and/or the bioproduct's natural equivalent, if feasible. Manufacturers should also present suitably characterized in-house reference materials at the time of submission. These reference materials are intended for analytical (physicochemical and biological) assays of production lots. An in-house primary reference material is an appropriately characterized material prepared by the manufacturer from a representative lot(s) for the purpose of biological assay and physicochemical testing of subsequent lots, and against which in-house working reference material is calibrated. In contrast, an in-house working reference material is a material prepared similarly to the primary reference material that is established solely to assess and control subsequent lots for the individual attribute in question. It is always calibrated against the in-house primary reference material (EMEA's guidelines https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-6-b-test-procedures-acceptance-criteria-biotechnological/biological-products-step-5_en.pdf).

A range of analytical methods are employed to characterize the **physicochemical properties**: composition, physical properties, and primary structure (desired secondary, tertiary etc. structure is commonly confirmed based on biological activity). This set of techniques aims to uncover any changes that may occur in the structure of the desired bioproduct during manufacture or storage, or as a natural part of the bioprocess. An overview of the common approaches is given in Analytical methods to elucidate physicochemical properties are listed in Appendix 6.1. of EMEA's guidelines. To see the full text, visit https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-6-b-

test-procedures-acceptance-criteria-biotechnological/biological-products-step-5_en.pdf.

Table 3.3.2 Approaches typically used for structural characterization and confirmation, and evaluation of physicochemical properties of the desired product, drug substance and/or drug product.

Type of analysis	Characteristics
Structural characterization and confirmation	Amino acid sequence
	Amino acid composition
	Terminal amino acid sequence
	Peptide map
	Sulfhydryl group(s) and disulfide bridges
	Carbohydrate structure
Physicochemical properties analysis	Molecular weight or size
	Isoform pattern
	Extinction coefficient (or molar absorptivity)
	Electrophoretic patterns
	Liquid chromatographic patterns
	Spectroscopic profiles

Heterogeneity that occurs during manufacture and/or storage affects the quality of the bioproduct. This heterogeneity should have a consistent pattern, with consistency in product activity, efficacy and safety. Protein heterogeneity that is inherent to the bioprocess is often due to alternative post-translational modifications. What is important in this case is for the manufacturer to demonstrate that the resulting mixture of forms is safe (e.g. immunologically safe) and active, and that the pattern of heterogeneity is consistent. Any deviations from the pattern defined at the preclinical and clinical stages require additional testing.

Biological properties (i.e. biological activity) are assessed to demonstrate that the bioproduct has the ability/capacity to exert its desired biological effect. The manufacturer should provide a valid bioassay for this purpose. The most common types of bioassays and the endpoints they use are outlined in Table 3.3.3 .

Table 3.3.3 Types of assays commonly used for assessment of biological activity.

Biological assays	Endpoints
Animal-based assays	Experimental animals' biological response to the product
Cell-culture–based assays	Biochemical or physiological response at the cellular level
Biochemical assays	Biological activities (enzyme activity, immunological reaction, ligand/receptor binding, etc.)

The quantitative measure of biological activity is the **potency** of the bioproduct (versus **mass** in the case of physicochemical assays). Potency is the measure of the biological activity using a suitably quantitative biological assay (also called potency assay or bioassay), based on the attribute of the product which is linked to the relevant biological properties (EMEA's guidelines https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-6-b-test-procedures-acceptance-criteria-biotechnological/biological-products-step-5_en.pdf). The potency reflects the biological properties of the bioproduct. When the bioproduct is to be used as a drug, its activity in the biological assay should correlate with the expected clinical response. The units of activity measured in the biological assay should be calibrated against an international or national reference standard, or if such does not exist, against an in-house one. For bioproducts with complex higher order structure, the biological activity may be more informative than the physicochemical properties. There are only limited cases when physicochemical tests may replace a biological assay (Table 3.3.4).

EMEA requires the choice of relevant quantitative assay (biological and/or physicochemical) for the purpose of batch release to be justified by the manufacturer.

Table 3.3.4 Conditions justifying the substitution of biological assays with physicochemical tests.

Physicochemical tests can replace biological assay when:
Physicochemical techniques can provide sufficient information about the bioproduct, including its higher-order structure, and There is relevant correlation to its biological activity
The bioproduct has a well-established manufacturing history

Testing the **immunological properties** is essential when the bioproduct is an antibody (affinity, avidity and immunoreactivity) or a protein drug. In the case of antibodies, the tests are typically binding assays to purified antigens and defined regions of antigens are used. In the case of protein drugs, immunochemical assays are often applied (e.g., ELISA, Western-blot) to demonstrate the protein's identity, homogeneity or purity, or to quantify it. If the bioproduct's immunochemical properties are among the criteria for lot release, these properties should be made available.

Another important property of bioproducts that needs to be tested is their absolute and relative **purity**. The relative purity is defined based on specific activity, i.e. units of biological activity per milligram of product. Since it largely depends on the method of choice, EMEA's guidelines require that the purity of the drug substance/product be tested using a combination of analytical assays.

As pointed out above, bioproducts can include several molecular entities or variants, e.g. owing to intrinsic or manufacture/storage-related heterogeneity. Such variants are not considered impurities, but **product-related substances**, when they are an anticipated part of the desired product and their properties do not deviate from its specifications. Product-related substances are molecular variants of the desired product formed during manufacture and/or storage which

are active and have no deleterious effect on the safety and efficacy of the drug product. These variants possess properties comparable to the desired product and are not considered impurities (EMEA's guidelines https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-6-b-test-procedures-acceptance-criteria-biotechnological/biological-products-step-5_en.pdf). Product-related substances should meet pre-defined individual and/or collective acceptance criteria, as appropriate. For the purpose of lot release, purity should be determined based on an appropriate set of appropriately selected methods. A desired product is (1) the protein which has the expected structure, or (2) the protein which is expected from the DNA sequence and anticipated post-translational modification (including glycoforms), and from the intended downstream modification to produce an active biological molecule (EMEA's guidelines https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-6-b-test-procedures-acceptance-criteria-biotechnological/biological-products-step-5_en.pdf).

The manufacturer is also required to assess the **impurities**, which are defined as components that do not have properties comparable to those of the desired product with respect to activity, efficacy and safety. Impurities may be either process- or product-related (Table 3.3.5); of known structure, partially characterized or unidentified.

Table 3.3.5 Types of impurities based on origin.

Type of impurities	Origin	Examples
Process-related impurities	Generated during the manufacturing process or downstream processing	Cell substrates (e.g., host cell proteins, host cell DNA); Cell culture components (e.g., inducers, antibiotics, or media components)
Product-related impurities	Generated during manufacture and/or storage	Precursors Degradation products

EMEA requires that acceptance criteria (individual and/or collective) be set for impurities (product-related and process-related), as appropriate, based on data obtained from lots used in preclinical and clinical studies and manufacturing consistency lots. For the purpose of lot release, impurities should be reported based on an appropriate subset of analytical methods. Examples of analytical procedures which may be employed to test for the presence of impurities are listed in Appendix 6.1. of EMEA's guidelines. To see the full text, visit https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-6-b-test-procedures-acceptance-criteria-biotechnological/biological-products-step-5_en.pdf .

Contaminants are defined as all adventitiously introduced materials not intended to be part of the manufacturing process (1). For example, contaminants include chemical and biochemical materials (e.g., microbial enzymes), and/or microbial species. Contaminants must be strictly avoided and/or controlled based on suitable in-process acceptance criteria or action limits. An action limit is an internal (in-house) value used to assess the consistency of the process at less

critical steps (EMEA's guidelines https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-6-b-test-procedures-acceptance-criteria-biotechnological/biological-products-step-5_en.pdf). Action limits do not apply to certain contaminants, e.g. viruses or mycoplasma, so the strategies proposed in ICH Harmonised Tripartite Guidelines "Quality of Biotechnological/Biological Products: Viral Safety Evaluation of Biotechnology Derived Products Derived from Cell Lines of Human or Animal Origin" and "Quality of Biotechnological/Biological Products: Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products" should be considered. The control of contaminants is, naturally, also essential in food safety. An example case scenario for validation of cleaning and disinfecting protocols is given in Annex I of CAC's Guideline for the validation of food safety control measures (CAC/GL 69), as follows:

EXAMPLE: VALIDATION OF CLEANING AND DISINFECTING PROTOCOLS (Sanitation Standard Operating Procedures, SSOPs)

1. Pre-validation Tasks
    a. Hazard(s): Generic microbial pollutants
    b. Food Safety Outcome: Effective sanitation of food-contact surfaces as demonstrated by compliance with microbiological criteria.
    c. Control Measure(s): Cleaning and disinfection protocols (SSOPs) within a facility
2. Approach: Collection of scientific data.
3. Parameters and Decision Criteria: SSOPs will be considered to be validated if, after implementation of cleaning and disinfection protocols, food contact surfaces meet microbiological criteria established for aerobic plate counts or other indicator microorganisms as appropriate.
4. Assemble the relevant validation information
    a. SSOPs will be implemented as intended for 3-4 weeks of operation.
    b. Microbiological testing of food contact surfaces will be conducted after cleaning and disinfection protocols have been used at the end of each day's production.
5. Analyze the results
    a. Compare results obtained at the end of each day's production to the established microbiological criteria.
    b. Conduct appropriate statistical analyses to determine the variability in efficacy of the cleaning and disinfection procedures.
6. Document and review the validation
    a. Data from implementation of SSOPs should be documented.
    b. All data from food contact surface testing should be documented.
7. Conclusion

If review and analysis of the validation results indicate that the SSOPs are capable of consistently delivering results that comply with the established microbiological criteria during 3-4 weeks of the validation period, then the cleaning and disinfection protocols can be considered validated.

This same protocol with a reduced rate of testing can be used as an ongoing verification activity that the SSOPs are being implemented properly.

Another essential property of bioproducts is their **quantity**. A typical quantitative measure is protein content. The methods used for quantitative analysis are usually physicochemical ones. If the obtained quantity values directly correlate with the values obtained in the biological assay, it is possible to use measurement of quantity instead of the measurement of biological activity in the manufacturing processes.

3.3.3 Reference standards and validation of analytical procedures

Reference standards serve to test production lots. In the context of food safety, the control of hazards potentially associated with foods typically involves the application of control measures in the food chain, from primary production, through processing, to consumption. The *Guidelines for the validation of food safety control measures* of the Codex Alimentarius Commission present information on the concept and nature of validation, tasks prior to validation, the validation process and the need for re-validation. The difference between validation, monitoring and verification is addressed in CAC's Guideline for the validation of food safety control measures (CAC/GL 69). For examples of validation of food safety control measures, see Annex I of CAC's Guideline for the validation of food safety control measures (CAC/GL 69).

EMEA (1) requires manufacturers to provide a reference standard at the time of submission. This should either be an already established international or national standard, or in the case of new molecules, an in-house primary **reference material** with known characteristics used for calibration purposes. Typically, the same reference material is used in different types of tests (e.g. biological activity, physicochemical characteristics, purity), although if necessary, it is possible to use separate reference materials (e.g. for impurities of different origin). The application procedure also requires the manufacturer to provide a description of the manufacture and/or purification of reference materials (if applicable), along with documentation of the characterization, storage conditions and stability of reference material(s).

Another key step is the **validation** of the analytical procedures in the specifications. According to EMEA's guidelines (1), it needs to be done prior to submission of the application to the regulatory authorities and must follow the applicable guidelines. These are the ICH Harmonised Tripartite Guidelines "Validation of Analytical Procedures: Definitions and Terminology" and "Validation of Analytical Procedures: Methodology". Exceptions are possible in cases when the analysis of certain biotechnological and biological products requires unique tests. Regarding food derived from modern biotechnology, food authentication, food speciation etc., the Codex Alimentarius Commission places an emphasis on the acceptance of methods of analysis which have been validated through a collaborative trial conforming to an internationally accepted protocol according to ISO 5725:1994 or the AOAC/IUPAC Harmonized Protocol.

3.3.4 Process control: from early development through commercial scale production

Process control is a prerequisite to the reproducibility of any industrial manufacture of bioproducts according to specifications. Accurate process design and description of process limitations are a must. The limits are defined on the basis of critical data collected at all stages: from initial development to large-scale production. This guarantees that the manufactured bioproduct will meet all its specifications.

Control of impurities is an important aspect. However, there are certain impurities that may not require testing of the drug substance or the drug product, given that the manufacturer demonstrates efficient control or removal to acceptable levels in line with specifications.

- ✓ A ***drug substance*** is the material which is subsequently formulated with excipients to produce the drug product. It can be composed of the desired product, product-related substances, and product- and process-related impurities. It may also contain excipients including other components such as buffers (EMEA's guidelines https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-6-b-test-procedures-acceptance-criteria-biotechnological/biological-products-step-5_en.pdf).
- ✓ A ***drug product*** is a pharmaceutical product type that contains a drug substance, generally, in association with excipients (EMEA's guidelines https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-6-b-test-procedures-acceptance-criteria-biotechnological/biological-products-step-5_en.pdf).

EMEA's guidelines also take into consideration the fact that only limited data may be available at the time of submission, and may in some cases allow such data to be supplemented after marketing authorization, in accordance with regional regulations.

Process control aims to guarantee consistency of the production process. There are two aspects to process control: defining **action limits** and **acceptance criteria**. Firstly, process control is achieved through in-process tests at certain critical steps (or less critical ones) in order to make decisions based on action limits (e.g. investigate or take certain action). Action limits are to be set by the manufacturer on the basis of data gathered during development and validation runs and are subject to further refinement after product approval. Secondly, in-house testing generates data that are compared against relevant acceptance criteria. For bioproducts, for instance, acceptance criteria need to be defined for in-process testing for adventitious agents at the end of cell culture. In some cases in-house tests may offer sufficient evidence of proper adherence to specifications so as not to require testing of the drug substance or the drug product, as mentioned above.

Control must also be done for the quality of the raw materials and excipients according to relevant standards. An excipient is an ingredient added intentionally to the drug substance which should not have pharmacological properties in the quantity used (EMEA's guidelines https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-6-b-test-procedures-acceptance-criteria-biotechnological/biological-products-step-5_en.pdf). For example, if the bioproduct is intended for human use (e.g. drug substance or drug product), the raw biomaterials or reagents need to be tested

for the presence/absence of potentially harmful **endogenous** or **adventitious agents**. If some steps of the manufacture include monoclonal antibodies (e.g. affinity chromatography), measures need to be taken to guarantee that the quality and safety of the product are not compromised by any related impurities or potential contaminants. The quality of the excipients and the container/closure systems is defined in pharmacopoeias. For non-pharmacopoeial excipients, appropriate acceptance criteria should be established.

Pharmacopoeial specifications refer to certain analytical procedures and acceptance criteria, which are applied for evaluation of a drug substance or drug product. Examples of pharmacopoeial specifications for bioproducts include tests for sterility, endotoxins, microbial limits, volume in container, uniformity of dosage units, particulate matter etc. The pharmacopoeial methods and acceptance criteria define guidance values for harmonization so that the analytical procedures and acceptance criteria are identical or methodologically equivalent.

Release limits or **shelf-life limits** may be established where justified, e.g. potency and degradation products. The release limits are tighter the shelf-life limits of the drug substance or drug product.

Appropriate **statistical analysis** of quantitative results is required, when necessary. The choice and rationale of statistical methods should be justified and described in sufficient detail to allow independent calculation.

3.3.5 Quality characteristics – reasoning of the specification

The reasoning behind the need to set specifications for drug substance and drug product lies in the fact that they are part of the control strategy that guarantees the appropriate quality of each lot (See Box). In other words, specifications serve to confirm the quality, and not to characterize the product. The manufacturer is expected to provide the rationale and justification for including and/or excluding certain tests for specific quality attributes. This justification needs to be based on scientifically sound considerations.

Box 3.3.1 Specifications serve as assurance that the quality attributes are maintained.

Specifications apply to all aspects of the manufacturing process:

- control of raw materials and excipients
- in-process testing
- process evaluation or validation
- adherence to GMP (Good Manufacturing Practices)
- stability testing
- testing for consistency of lots

The following points should be taken into consideration when establishing scientifically justifiable specifications.

- ✓ Specifications are linked to a manufacturing process.

Specifications are used to demonstrate manufacturing consistency, specifically for **product-related substances**, **product-related impurities** and **process-**

related impurities. Product-related impurities are molecular variants of the desired product (e.g., precursors, certain degradation products arising during manufacture and/or storage) which do not have properties comparable to those of the desired product with respect to activity, efficacy, and safety (EMEA's guidelines https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-6-b-test-procedures-acceptance-criteria-biotechnological/biological-products-step-5_en.pdf). Process-related substances are impurities that are derived from the manufacturing process. They may be derived from cell substrates (e.g., host cell proteins, host cell DNA), cell culture (e.g., inducers, antibiotics, or media components), or downstream processing (e.g., processing reagents or column leachables) (EMEA's guidelines https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-6-b-test-procedures-acceptance-criteria-biotechnological/biological-products-step-5_en.pdf).

Heterogeneity patterns other than those specified during preclinical and clinical development need investigation. Specifications should be based on data obtained from lots used to demonstrate manufacturing consistency. Linking specifications to a manufacturing process is important, especially for product-related substances, product-related impurities and process-related impurities. Process changes and degradation products produced during storage may result in heterogeneity patterns, which differ from those observed in the material used during preclinical and clinical development. The significance of these alterations should be evaluated.

- ✓ Specifications should account for the stability of drug substance and drug product.

Specifications should take into account changes in the quality due to degradation during storage. Since bioproducts are so diverse, their stability characteristics cannot be assessed using a universal stability indicator. The manufacturer should propose a product-specific stability-indicating profile. Degradation of drug substance and drug product, which may occur during storage, should be considered when establishing specifications. Due to the inherent complexity of these products, there is no single stability-indicating assay or parameter that profiles the stability characteristics. Consequently, the manufacturer should propose a stability-indicating profile. The result of this stability- indicating profile will then provide assurance that changes in the quality of the product will be detected. The determination of which tests should be included will be product-specific. The manufacturer is referred to the ICH Harmonised Tripartite Guideline: "Stability Testing of Biotechnological/Biological Products".

- ✓ Specifications are linked to preclinical and clinical studies.

Specifications should be based on data obtained for lots used in pre-clinical and clinical studies. Specifications should be based on data obtained for lots used in pre-clinical and clinical studies. The quality of the material made at commercial scale should be representative of the lots used in preclinical and clinical studies.

- ✓ Specifications are linked to analytical procedures.

There are multiple analytical procedures used for **critical quality attributes** (e.g. potency, the nature and quantity of product-related substances, product-related impurities and process-related impurities). The analytical technology may evolve in the course of product development, so the data obtained during development should correlate with the data obtained at the time the marketing application is filed. Critical quality attributes may include items such as potency, the nature and quantity of product-related substances, product-related

impurities, and process-related impurities. Such attributes can be assessed by multiple analytical procedures, each yielding different results. In the course of product development, it is not unusual for the analytical technology to evolve in parallel with the product. Therefore, it is important to confirm that data generated during development correlate with those generated at the time the marketing application is filed.

The battery of tests that get included in the specifications depends on each specific product. The rationale behind the definition of acceptable range of acceptance criteria should be justified based on data obtained from lots used in preclinical and/or clinical studies, data from lots used for demonstration of manufacturing consistency, and data from stability studies, and relevant development data. In some cases, testing at production stages may be sufficient. In some cases, testing at production stages rather than at the drug substance or drug product stages may be appropriate and acceptable. In such circumstances, test results should be considered as in-process acceptance criteria and included in the specification of drug substance or drug product in accordance with the requirements of the regional regulatory authorities.

The tests and acceptance criteria that apply to **drug substance specifications** are outlined in Table 3.3.6. Pharmacopoeial tests (e.g., endotoxin detection) should be performed on the drug substance, where appropriate. Additional drug-substance–specific acceptance criteria may also be necessary.

Table 3.3.6 Drug substance specifications

Appearance and description	**Physical state (e.g., solid, liquid)** **Color**
Identity	Tests are: qualitative drug-substance–specific based on unique properties (e.g. molecular structure) Several tests are often necessary (physicochemical, biological and/or immunochemical). Some of the methods typically used for characterisation of the product as described in Appendix 6.1 of EMEA's guidelines may be employed and/or modified as appropriate for the purpose of establishing identity. To see the full text, visit https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-6-b-test-procedures-acceptance-criteria-biotechnological/biological-products-step-5_en.pdf

Purity and impurities	Absolute purity of bioproducts is difficult to determine Results are method-dependent A combination of methods is used Analytical procedures are focused on separation of desired product from product-related substances and from impurities. Impurities can be process- or product-related. **Process-related impurities** in the drug substance may include cell culture media, host cell proteins, DNA, monoclonal antibodies or chromatographic media used in purification, solvents and buffer components. These impurities should be minimised by the use of appropriate well-controlled manufacturing processes. **Product-related impurities** in the drug substance are molecular variants with properties different from those of the desired product formed during manufacture and/or storage. Under certain circumstances, acceptance criteria for selected impurities may not be required. Individual and/or collective acceptance criteria for impurities should be set
Potency	A relevant, validated potency assay An alternative method (physicochemical and/or biological) may be sufficient for quantitative assessment Specific activity may provide additional useful information in some cases
Quantity	Usually determined based on protein content (mass) Quantitative analysis may be independent of a reference standard or material Potency assay may be sufficient in some cases

The tests and acceptance criteria that apply to **drug product specifications** correspond to those that apply to drug substances (Table 3.3.1).

Table 3.3.7 Drug product specifications

Appearance and description	**Physical state (e.g., solid, liquid)** **Color** **Clarity** **pH** **Osmolarity**
Identity	Tests are: qualitative drug-product–specific based on unique properties (e.g. molecular structure) One or more tests are often necessary (physicochemical, biological and/or immunochemical). Some of the methods typically used for characterisation of the product as described in Appendix 6.1 may be employed and/or modified as appropriate for the purpose of establishing identity.
Purity and	Impurities:

impurities	may be generated or increased during manufacture and/or storage may be the same as those in the drug substance, process-related, or formed in the drug product during formulation or storage Testing is not necessary if impurities are qualitatively and quantitatively the same as in the drug substance Testing and acceptance criteria apply if impurities are known to be introduced/formed during production and/or storage Acceptance criteria and analytical procedures based on previous experience with the drug product Analytical procedures are chosen and optimized to separate the desired product and product-related substances from impurities and excipients
Potency	Relevant, validated potency assay An alternative method (physicochemical and/or biological) may be sufficient for quantitative assessment when an appropriate potency assay is used for the drug product (rationale for the choice of method is needed)
Quantity	Usually determined based on protein content (mass) Potency assay may be sufficient if manufacture is based on potency

Pharmacopoeial tests (sterility, endotoxins, microbial limits, volume in container, particulate matter, uniformity of dosage units, moisture content etc.) apply to the relevant dosage forms. The tests for uniformity of dosage units may be done as in-process controls and corresponding acceptance criteria set. Some unique dosage forms may need additional tests other than those outlined in (Table 3.3.1).

With reference to quality assurance of food control laboratories, special attention is paid to ensure that the consumers get a safe, wholesome food product free from adulteration, correctly labelled and presented. A Codex standard for any food or foods should be drawn up in accordance with the Format for Codex Commodity Standards (2). The Codex Alimentarius Commission has also adopted *Harmonized Guidelines for Internal Quality Control in Analytical Chemistry Laboratories*. They are particularly relevant for the application of molecular and immunological analytical methods which are currently recognized as tools for determination of specific DNA and protein analytes in foods.

3.3.6 References

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Course 3.4: Environmental assessment of bioproducts: towards sustainable production process

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3.4.1 Introduction

Bioproducts are often considered more environmentally-friendly than their conventional counterparts owing to biodegradability, biocompatibility and sustainability. Better utilization of side streams and low value fish can simultaneously improve both the environmental and ecological sustainability of production. Nonetheless, there are a number of environmental considerations associated with the production of bioproducts that need to be taken into account. For example, the environmental hazards and risks associated with fisheries and aquaculture can be broken down into biological pollution, organic pollution, chemical pollution and habitat modification (Table 3.).

'Hazards' refers to sources of potential harm, whereas 'risk' considers the frequency and severity of damage from hazards (World Bank, http://siteresources.worldbank.org/INTSAFEPOL/1142947-1116493361427/20507357/Update21EnvironmentalHazardAndRiskAssessmentDecember1997.pdf).

Table 3.4.1 Environmental hazards and risks associated with aquaculture

Hazards/risks	Cause	Effect
Biological pollution	• Accidental release of non-endemic or genetically modified species into natural water • Exotic fish species outcompeting native stocks due to the lack of natural predators, parasites and pathogens in the new environment • Inter-breeding of exotic or transgenic fish with the native stock	• Transmission/introduction of non-endemic diseases and parasites • Biodiversity loss in natural waters • Genepool dilution • Negative impact on key ecological processes
Organic pollution	• Effluents from aquaculture facilities (fish and feed wastes) that contain large quantities of nutrients	• Damaged water quality • Unwanted algal growth • Negative impact can be mitigated via utilization of wastes/by-products for bioproducts
Chemical pollution	• Chemicals used in ponds and laboratories (fertilizers, pesticides, antifoulants, fungicides, etc.)	• Pollution of natural aquatic systems • Health and food safety concerns
Habitat modification	• Changes in landscapes of aquatic systems due to construction of aquaculture sites	• Habitat destruction and loss of biodiversity • Disruption of natural ecological

		balance

Aquaculture, also called fish farming, fish culture, or mariculture, is the rearing of fish, shellfish, and some aquatic plants to supplement the natural supply (Encyclopaedia Britannica, https://www.britannica.com/topic/aquaculture). Fishery is the combination of a fishing area plus the fishing gear and a target species, as defined in BE-FAIR Project. Good practice manual for recovery, handling and classification of discards and by-products on fishing fleets and fish auctions, 2006 (ec.europa.eu). Failure to maintain adequate management in aquacultures and fisheries can bring about ecological and environmental problems. For example, the practice of throwing back into the sea the discards and by-products of on-board fish processing can lead to a change in the overall structure of marine trophic webs and habitats and/or spread of parasites present in fish viscera. Discards are the portion of the organic material of animal origin in the catch, which is thrown back into the sea either because of low value or legal requirements. Discards could be dead or alive. Fish offal is not included. By-products are solid or liquid parts of fish and other marine organisms, generated by the industrial processing and consisting of unprocessed fish and parts of fish, such as heads, tails, fins, scales, bones, blood, skins, flesh, roe, viscera, as well as the organic parts of other marine organisms (shellfish, shells…), which are not intended for human consumption.

In addition, subproducts and wastes originated in land during the industrial processing of fishery products are known to cause environmental, pollution and disposal problems.

According to Directive 2011/92/EU, "*environmental impact assessment shall identify, describe and assess in an appropriate manner, in the light of each individual case, the direct and indirect significant effects of a project on…*: *(a) population and human health; (b) biodiversity …; (c) land, soil, water, air and climate; (d) material assets, cultural heritage and the landscape; (e) the interaction between the factors referred to in points (a) to (d)"*.

Directive 2011/92/EU further defines 'environmental impact assessment' as "*a process consisting of:*

(i) the preparation of an environmental impact assessment report … ;

(ii) the carrying out of consultations …;

(iii) the examination by the competent authority of the information presented in the environmental impact assessment report and any supplementary information provided, where necessary, by the developer …, and any relevant information received through the consultations …;

(iv) the reasoned conclusion by the competent authority on the significant effects of the project on the environment, taking into account the results of the examination referred to in point (iii) and, where appropriate, its own supplementary examination; and

(v) *the integration of the competent authority's reasoned conclusion into any of the decisions…"*

This Unit is focused on the methodologies that are commonly used for environmental impact assessment (EIA) and some key regulations on environmental assessment associated with the production of biobased products (and specifically ones from fisheries and aquaculture).

3.4.2 Environmental assessment studies on biobased products: methodologies for impact evaluation

Environmental impact assessment (EIA) aims to identify, estimate and evaluate the environmental impacts of a project before its execution. The goal is to alleviate the potential negative effects before any liabilities are made. Systematic assessment and evaluation also covers the economic performance of the bio-based production, different environmental loads, etc. Environmental criteria are particularly important in the design and implementation of large-scale bio-based production systems, to take into account any negative environmental effects associated with all aspects of the transportation, storage and production activities. Comparative analysis of alternative types of feedstock and/or processes and technologies facilitates making the optimal choice. Box 3.4.1 outlines how environmental assessment studies can help optimize various aspects of bio-based systems prior to project execution, according to Balaman.

Box 3.4.1 Environmental assessment studies for bio-based systems, according to Balaman.

- Comparing environmental performance of different feedstock for different purposes (e.g., heat and/or power generation, bio-fuel production, production of pellets, etc.),
- Comparing environmental performance of different conversion technologies and scales,
- Estimating the environmental impacts of various operational strategies for biomass cultivation, harvesting, and collection,
- Rating the environmental impacts of different options for bio-product transportation vehicles, fuels, and operational strategies for logistic systems
- Evaluating the environmental performance of different bio-products; it can be used in standardization and certification of bio-based products,
- Assessing the environmental impacts of various alternatives for storage of bio-sources and bio-products,
- Comparing the environmental performance of different bio-sources preprocessing options,
- Comparing the environmental impacts of different material options for construction of biomass-based production, storage, and preprocessing plants,
- Providing a perspective on resource depletion and environmental emissions to the organs of state for support in making policies, plans, and proposing legislations and incentive schemes about biomass-based production systems.

The study or evaluation of environmental impact involves a set of steps/activities. They include screening, scoping, prediction and mitigation, management and monitoring, and audit. The methodologies that are applicable to each particular case depend on the objectives of the various activities. The definition of the term *methodology* adopted here is the one used by Canter: "*structured approaches for achieving one or more of the basic activities*".

Screening, or *impact identification*, aims to outline the most important changes (i.e. impacts) that a project is expected to cause in the environment. This step identifies how environmentally sensitive the project is based on the key sources of impact and types of impact, e.g. biological and physico-chemical, as well as

social, health and economic aspects. This will determine whether an EIA is needed and if so to what detail.

The specifics identified at the screening stage serve to determine the key environmental issues to be assessed (*scoping*) and, consequently, to select appropriate methodologies for the assessment. The identified problems serve as a basis for early mitigation measures and help target the subsequent steps of EIA at the most important issues. Scoping also identifies the key interest groups and any public concerns. The main EIA techniques used in scoping are baseline studies (data about the existing environment and environmental conditions), checklists, matrices and network diagrams (means of data representation).

Experts make *predictions* of the expected impact to help decision-makers take informed decisions and provide relevant information for the general public. The *assessment* stage focuses on the potential environmental and socio-economic effects of the project, and evaluates and compares the alternatives identified in the previous stages.

Alternative ways to *mitigate* (minimize) the effects through optimized design and environmental *management* are identified and compared. The technique used to assess the accuracy of predictions is *mathematical modeling*. The models should be appropriately chosen depending on the available data. For example, these could be ecological models to predict changes in aquatic biota as a result of toxic substance discharge. When there are limited/insufficient data, it is common practice to use *expert judgment*, or *expert advice*, to complement the modeling methods. The EIA results are typically presented using *checklists*, *matrices*, *network diagrams*, *graphical comparisons* and *overlays*, to allow easy interpretation of results and comparison of alternatives.

For example, *Rapid Impact Assessment Matrix (RIAM)* is a systematic approach using qualitative data that can be expressed in a semi-quantitative way. In this method a multidisciplinary team performs analysis of physico-chemical, biological, human and economic data in a common matrix. The resulting interactive impact profile allows the practitioner to make a rapid comparison of alternative options.

Finally, a summarized *EIA report* is produced, which is filed for review by the competent authority to receive approval for execution or recommendations for further improvements. Following approval for execution, there needs to be strict *monitoring* to make sure that the *Environmental Management Plan* is accurately implemented. By comparing the predicted and the actual impacts it is possible to identify problems early and take appropriate action. A flowchart of the EIA stages is shown in Figure 3.4.1. For more information about EIA, visit the International Institute for Sustainable Development (IISD) at https://www.iisd.org/learning/eia/.

An *Environmental Audit* is carried out some time after implementation/completion of the project. The audit includes analyses of the technical, procedural and decision-making aspects of the EIA. The purpose of the audit is to determine whether the recommendations and requirements in the EIA steps have been incorporated successfully into project implementation.

In EIA it is sometimes important to undertake *cumulative impact assessment* to take into account the cumulative impacts of multiple and successive environmental and social impacts, which may have an additive effect. Examples of cumulative impact are the increases in pollutant concentrations in a water body or their bioaccumulation in fish. Mitigation of cumulative impacts often

requires the collaborative efforts of different stakeholders, including governments.

Another popular methodology for conducting environmental impact analysis is the so-called *Battelle Environmental Evaluation System*. It is based on a hierarchical assessment of environmental quality indicators. Product environmental *life cycle analysis (LCA)* is often applied to identify and quantify the impact of industrial-scale biomass-based systems and production chains on the environment, e.g. projects for generation of bio-fuel, bio-chemicals and other bio-products. Specific methods have been developed for EIA of genetically modified organisms. More complicated approaches for EIA based on fuzzy logic are also being explored. For more information on the Battelle Environmental Evaluation System, see http://ponce.sdsu.edu/the_battelle_ees.html. To read a Wikipedia article on EIA, visit: https://en.wikipedia.org/wiki/Environmental_impact_assessment.

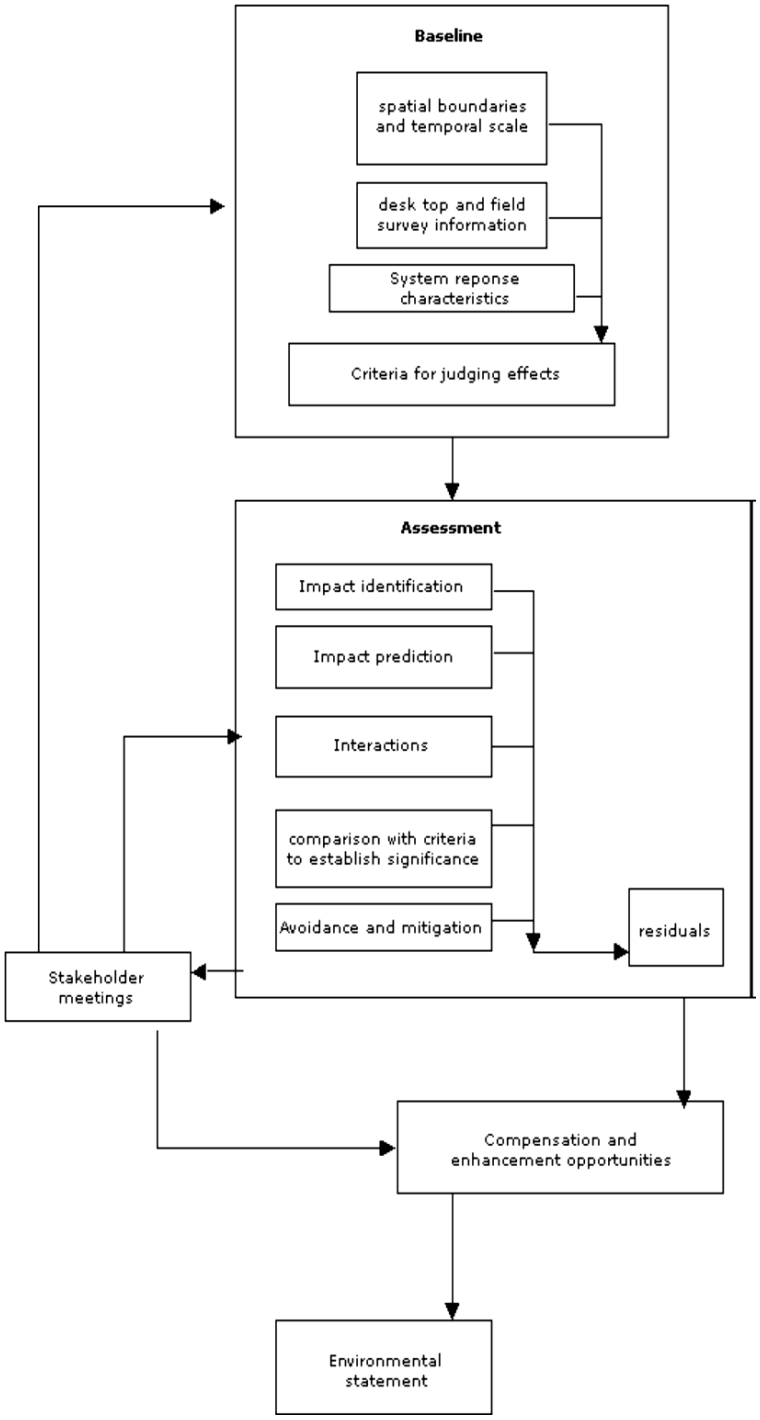

Figure 3.4.1 EIA stages according to Environmental Impact Assessment (EIA) and Environmental Impact Statement (EIS). UNESCO.

Recent advancements in biotechnology have contributed towards more sustainable aquaculture and a greater diversity of bioproducts of marine origin. This is achieved owing to better assessment of chemical and biological interactions between aquaculture and the environment, development of mitigation strategies, efficient microbial bioremediation processes and improved microbial control of intensive production systems.

Pond water quality needs to be well-managed and balanced by aquaculturists. For a summary of typical ranges of water-quality indicators for representative aquaculture water sources, effluents, and recovered aquaculture sludge versus

runoff waters, municipal sewage, and various industrial and agricultural wastes. An overview of coastal aquaculture-specific monitoring parameters and characteristics for which data may be collected is given in Annex 4 of the FAO Guidelines for the Promotion of Environmental Management of Coastal Aquaculture Development.

3.4.3 Regulatory aspects of environmental assessment – biological and chemical hazards

It is common knowledge that there are certain biological and chemical hazards associated with animal by-products, whether disposed of or utilized with new value added applications. The organic components of fish waste (flesh, skin bones, etc.) have a high biological oxygen demand and, if not managed properly, can pose environmental and health problems. Fish waste includes the by-catch and by-products produced either on board, in land and by industries. Also the incidental catch when it is dead.

- ✓ By-catch: The unintentional capture of non-target fish species.
- ✓ Fish: Includes all fishes and other aquatic species such as crustaceans, cephalopods and mollusks.
- ✓ Incidental catch: Rare events as catches of marine mammals, turtles or seabirds. (BE-FAIR Project. Good practice manual for recovery, handling and classification of discards and by-products on fishing fleets and fish auctions, 2006 (ec.europa.eu).)

It is well recognized that waste can be reduced by utilization of animal by-products. However, this also involves the handling (the manner of elaborating fish and removing different by-products of each specimen (head, viscera, tails, fins, liver)) of significant amounts of hazardous materials, e.g. raw materials and intermediate/final products. For example, the processing steps may involve certain chemical hazards; animal by-products and bioproducts derived from by-products might be a vector for the transmission of microbial or viral illnesses. Therefore, the disposal, transport, storage and handling of such materials must be managed properly to avoid or minimize the environmental impacts.

The management and control of hazards (biological and chemical ones) in aquaculture requires specific strategies, including the identification of hazard, control of the hazard and monitoring of the effectiveness of the controls. Governments need to enforce proactive policies and legislation towards EIA to ensure problem prediction and preventive measures. There should be clear-cut guidelines on how to achieve a basic level of environmental protection in the vicinity of aqua farms.

In the EU, environmental impact assessment (EIA) is regulated by the so-called **EIA Directive**, its current version being Directive 2014/52/EU. The EIA Directive aims to ensure that a high level of environmental protection is achieved and that environmental considerations are integrated into the preparation and authorization of projects. Historically, the first version of the EIA Directive (85/337/EEC) came into force in 1985, applying to a wide range of defined public and private projects. The EIA Directive of 1985 was amended three times: in 1997 (Directive 97/11/EC), in 2003 (Directive 2003/35/EC) and in 2009 (Directive 2009/31/EC). The initial Directive of 1985 and its three amendments have been codified by Directive 2011/92/EU of 13 December 2011. Directive

2011/92/EU was amended in 2014 by Directive 2014/52/EU. The latest amendments aim to achieve better optimized administrative process, improved level of environmental protection together with sustainable business decisions on public and private projects, and relevant consideration of newly emerged threats and challenges, adding focus on resource efficiency, climate change, disaster prevention and so on. The main amendments are listed in Box 3.4.2. For detailed information, see Review of the EIA Directive https://ec.europa.eu/environment/eia/review.htm. For an informal consolidated version of the EIA Directive, see https://ec.europa.eu/environment/eia/pdf/EIA_Directive_informal.pdf. For a document summary, see: https://eur-lex.europa.eu/legal-content/EN/LSU/?uri=CELEX:32014L0052.

Box 3.4.2 Main amendments in Directive 2014/52/EU (21; 7).

- EU countries can simplify their different environmental assessment procedures.
- Timeframes are introduced for the different stages of environmental assessments.
- The screening procedure, determining whether an EIA is required, is simplified.
- Decisions must be duly motivated in the light of the updated screening criteria.
- EIA reports are to be made more understandable for the public, especially as regards assessments of the current state of the environment and alternatives to the project in question.
- The quality and the content of the reports is improved. Competent authorities also need to prove their objectivity to avoid conflicts of interest.
- The grounds for development consent decisions must be clear and more transparent for the public.
- If projects do entail significant adverse effects on the environment, developers are obliged to avoid, prevent or reduce those effects. These projects must be monitored.

The EIA Directive classifies all projects into Annex 1 or Annex 2 projects. Annex 1 includes large-scale projects (e.g. motorways, bridges, power stations etc.), which always require EIA. Annex 2 encompasses projects of a smaller scale, which could be subject to EIA according to the criteria laid out in Annex 3, as determined by each Member State. See also: https://en.wikipedia.org/wiki/Environmental_impact_assessment#Methods.

The EIA Directive covers all the steps of the EIA process. At the scoping stage, the project developer may request the competent authority to specify what should be covered by the EIA information to be provided. The developer is required to provide an EIA report drafted in accordance with Annex IV of the Directive. The environmental authorities (including local and regional ones as well as other affected EU countries) and the public must be informed and consulted. The competent authority makes a decision based on the results of consultations and the conclusions about the significant effects of the project. The authority informs the public of its decision. Public consultations are an essential part of EIA process. Effective public participation must be ensured by providing the EIA report and other information electronically, by public notices, or via local newspapers, etc. The public can challenge the decision before the courts. EU countries may lay down more stringent conditions and fix penalties for any infringements.

The transboundary aspects of EIA are specifically laid out in the Convention on Environmental Impact Assessment in a Transboundary Context (the so-called

Espoo Convention), which was signed by the European Community on 25 February 1991 and was ratified on 24 June 1997. According to this Convention, member states are obliged to carry out EIA of certain activities at an early stage of planning and to notify and consult each other on all major projects that are expected to have significant adverse environmental impact across boundaries. The Convention has had several amendments. More details are outlined in the following Wikipedia article: https://en.wikipedia.org/wiki/Convention_on_Environmental_Impact_Assessment_in_a_Transboundary_Context.

Specific guidelines for assessment of fish waste, or material resulting from industrial fish processing operations are laid out in the Convention on the Prevention of Marine Pollution by Dumping of Wastes and Other Matter, or the so-called London Convention. This Convention is one of the first international conventions for the protection of the marine environment from human activities. It came into force on 30 August 1975 and was followed by amendments and the so-called London Protocol of 1996, which entered into force in 2006. The London Protocol takes an approach that prohibits all dumping, except for a "reverse list" of possibly acceptable wastes. Dumping is the disposal into the sea of fish waste or organic materials resulting from the processing activities on board of vessels (BE-FAIR Project. Good practice manual for recovery, handling and classification of discards and by-products on fishing fleets and fish auctions, 2006 (ec.europa.eu).

Another EU policy document that is focused on the protection of water resources (fresh and water ecosystems and drinking water) is the 'Blueprint to safeguard Europe's water resources' (COM/2012/0673). Data about impact on biodiversity are reported by EU Member States under the Marine Strategy Framework Directive (MSFD). The new Common Fisheries Policy (CFP) is a set of rules for managing European fishing fleets and for conserving fish stocks to guarantee stable, secure and healthy food supply for the long term.

The control of biological and chemical hazards associated with the usage and disposal of fish by-products is governed by the regulations applying to animal by-products. Regulation (EC) No 1069/2009 aims to prevent any risk from animal by-products to public and animal health or the environment. It acknowledges that "*Disease outbreaks could also have negative consequences for the environment, not only due to the disposal problems posed, but also as regards biodiversity*". The Regulation controls the collection, transport, storage, handling, processing and use or disposal of animal by-products. It encourages the utilization of by-products for other purposes (if safe), as an alternative to disposal whenever possible. If animal by-products need to be disposed of, this should be done in accordance with the associated risk, as set in the environmental legislation on landfilling (Council Directive 1999/31/EC) or industrial emissions in the case of incineration (Directive 2010/75/EU). In order to prevent potentially detrimental effects for the environment, the export of animal by-products and derived products intended for disposal by incineration and by landfill should be prohibited. The export of animal by-products and derived products should also be prevented where the objective is to use them in a biogas or composting plant to third countries which are not members of the Organization for Economic Cooperation and Development (OECD), in order to prevent potentially adverse environmental impacts and health risks.

Commission Regulation (EU) No 142/2011 also covers the disposal (by incineration or landfilling) and use of animal by-products and derived products;

restrictions on the use of animal by-products and derived products; hygiene requirements for processing plants and other establishments, e.g. in the case of transformation of animal by-products and derived products into biogas and composting; and some derogations from certain provisions of Regulation (EC) No 1069/2009.

To prevent transmission of diseases, Commission Regulation (EC) No 1234/2003 does not allow feeding farmed animals intended for food with processed animal proteins with the exception of fishmeal, hydrolysed proteins and dicalcium phosphate for feeding of non-ruminants or fur-producing animals, upon certain conditions.

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