

Valuable applications for peat moss

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Abstract Peat is commonly known as a biofuel but it also has other more or less traditional uses, for example in folk medicine, building materials, or preservation of foods. Several studies suggest that the main composers of peat lands, *Sphagnum* mosses, may also have potential for a variety of other value-added products. Typically, those are related to the antibacterial and other preservative properties of *Sphagnum*, or to their high water adsorption ability. Molecular level applications, however, are still rarely reported. This might owe to the complex chemistry of *Sphagnum* and lacking cost-efficient technologies for the isolation of components of interest. In this literature survey, the structural and chemical properties of *Sphagnum* are reviewed together with their suggested uses. This is expected to facilitate new efforts to find commercially feasible applications for these extraordinary plants.

Keywords *Sphagnum* · Moss · Peat · Peat moss · Composition · Hydrolysis · Application · Fermentation · Preservation · Absorption

1 Introduction

Sphagnum mosses are globally important plants due to their ability to form peat and to bind atmospheric carbon dioxide [1–3]. Calculations suggest that there is more carbon locked up in *Sphagnum* biomass than is fixed by all terrestrial

vegetation in 1 year [4]. Accordingly, they are the main composers of boreal peat lands; without the genus, boreal peat lands would have neither their extent nor their particular features [5]. Peat lands are located in Alaska, northern Canada, northern Europe, and Siberia [2]. They cover globally an area of approximately 4 million square miles [6]. In Finland, peat lands cover one third of the total area (10,000,000 ha) holding an energy capacity above 100 TWh, and their renewing rate is approximately 50,000 ha in 50 years [7].

Sphagnum mosses are extraordinary plants, especially adapted to acidic, cool, waterlogged, and nutrient-poor conditions. They create themselves an acidic and anoxic environment which favors their growth. *Sphagnum* tolerate and require only low concentrations of dissolved nutrients, are resistant to decay, and are able to occupy various environments in peat lands [8]. In the tundra, *Sphagnum* mosses compose the majority of biomass, covering up to 100 % of the surface [9]. Damp environment is vital for *Sphagnum* moss plants, and they grow in boreal ecosystems that are characterized by rather cold climate and high annual precipitation [10, 11]. The genus *Sphagnum* comprises of 250–450 species from which only 50 are important peat formers [2, 10, 12].

Sphagnum mosses belong to bryophytes aka non-vascular plants. Thereby, they do not have true vascular tissues, i.e., their vascular tissues do not contain lignin. They do not have flowers or seeds and they reproduce via spores [13]. *Sphagnum* mosses grow from the top through a stem elongation. *Sphagnum* leaves are one cell thick with the majority of volume (ca. 90 %) comprising of dead, long, empty, thin-walled, and pored hyaline cells. Those are the channel for *Sphagnum* mosses to absorb water and nutrients from the atmosphere and water table [4]. Chlorophyllous cells of *Sphagnum* are embedded in between the hyaline cells.

Sphagnum mosses form very close-knit masses and lawns or floating mats. Those moss mats often form hummocks with

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hollows in between. Different *Sphagnum* species have different tolerances of pH, light, and wetness, and thus some species prefer well-drained hummocks while others prefer wetter hollows [14]. *Sphagnum* has been divided into ten different groups according to plant cell anatomy, the number of branches in a fascicle, the branch shape and color, and the plant habitat preference [13]. Many *Sphagnum* species have distinguishing red or brown colors which make the identification between different species difficult [8].

Sphagnum peat lands are formed of two layers, the acrotelm and catotelm [15]. The surface layer acrotelm is 10–40-cm thick and has a structure of a dense floating *Sphagnum* mat. This layer is water permeable although the water velocity is only in maximum 1 cm/s, and the lateral water transport is thus limited. The bulk density of *Sphagnum* moss increases from 0.02 to 0.1–0.2 g/cm³ when going from the acrotelm to catotelm. The catotelm layer is a thick, water-saturated, slowly permeable layer located under the acrotelm, in which the water velocity is even as low as 10⁻⁷–10⁻³ cm/s. Because of the slow permeability, the vertical water transport of mineral and nutrients are practically blocked in the catotelm. Therefore, the upper layers of *Sphagnum* moss are isolated from groundwater [15].

Peat deposits may be up to 6 m thick and even 10,000 years old [4, 10]. Peat formation starts when older part of *Sphagnum* plants die and collapse down [10]. In the base of the acrotelm, dead parts of *Sphagnum* mosses bend down and become compressed. In the catotelm, dissolved oxygen is exhausted and the slow anaerobic decomposition of dead parts of *Sphagnum* plants takes place [15].

The antimicrobial properties of living *Sphagnum* plants and *Sphagnum* peat have been known for centuries. A remarkable example is the existence of “bog bodies” which are human remains buried in peat and remained for centuries without significant decomposition. *Sphagnum* species are very resistant to microbial decay, and their decomposition is extremely slow. The suggested reasons for these properties include acidification of environment, low annual temperature and low nutrition in *Sphagnum*-dominated areas, anoxic conditions in *Sphagnum* litter, and antibiotic properties of the plant itself. Although there is still a debate on such mechanisms in *Sphagnum*, it is evidently an abundant and renewable source for organic compounds that could add value to current energy-focused use of peat [16].

Furthermore, the carbon emissions from use of *Sphagnum* would be lower than those of wood [17], and *Sphagnum* can actually be cultivated in areas where agriculture is generally not feasible [18]. The logistics for biomass already exist in former peat production areas. However, to facilitate new applications for *Sphagnum*, further investigations of their preprocessing and fractionation are required. In the following chapters, the current knowledge on *Sphagnum* properties and their utilization are reviewed, and potential new uses are discussed.

2 Composition of *Sphagnum*

Based on elemental, spectral, and non-destructive analytical methods, bryophytes consist mainly of carbohydrates, the most abundant functional groups being carbonyl, carboxyl, and hydroxyl groups. *Sphagnum* leaf cell walls have been suggested to contain polysaccharides similar to those of higher plants. In a study of monosaccharide linkages the most abundant was 4-Glc, typical of cellulose, but there was also evidence for xyloglucans, 4-linked mannans, 4-linked xylans, and rhamnogalacturonan-type polysaccharides [19]. The main composition of bryophytes and monosaccharide composition of *Sphagnum* are presented in Tables 1 and 2, respectively. According to elemental composition analysis, *Sphagnum* mosses contain approximately 41–42 % carbon, 5.5–5.8 % hydrogen, 0.4–1 % nitrogen, and 51–53 % oxygen.

Sphagnum mosses are able to absorb atmospheric nitrogen using symbiotic organisms such as cyanobacteria. These are present on *Sphagnum* plant leaves and convert atmospheric nitrogen into ammonia which is then utilized for the synthesis of amino acids [20]. The amount of nitrogen in *Sphagnum* is lower than in other bryophytes which may be due to growth environment [21].

The quantities of cellulose, hemicellulose, and lignin typically decrease during the decomposition of peat while the quantity of humic substances increases (Table 3). The proportions of bitumen (waxes, rosins) tend to increase or remain at a level of 1–5 %. The hemicellulose and cellulose contents of peat are approximately 47–68 and 57–76 % from that of moss, respectively [22]. The content of lignin-like phenolics, mostly humic acids, is 2–4 times higher in peat compared to moss. Accordingly, the most feasible application of peat depends on its decomposition degree.

2.1 Polysaccharides

Approximately 10–30 % of the dry mass of *Sphagnum* comprises of uronic acids, products of a reaction where the terminal group (CH₂OH) of a monosaccharide is oxidized chemically or biologically [4]. D-glucuronic acid, formed from D-glucose, is the major component of connective tissue polysaccharides. Among the *Sphagnum*, the uronic acid concentration is generally higher in hummock species compared to hollow species which also relates to the higher resistance to decay

Table 1 Main composition of bryophytes [21]

Component	Percent of dry weight [%]
Hemicellulose and pectin	30–60
Cellulose	15–25
Proteins	5–10
Lipids	5–10

Table 2 Monosaccharide composition of dried *S. papillosum* [29]

Component	Percent of dry weight [%]
Glucose	27.13
Galactose	5.87
Xylose	4.84
Mannose	4.39
Rhamnose	3.11
Arabinose	0.88
Fucose	0.3

among hummock species. *Sphagnum* mosses contain higher amounts of mannuronic acid, galacturonic acid, and especially glucuronic acid than more advanced mosses. These compounds may be related to the sponge-like properties of *Sphagnum* primary cell wall, enabling the primary cell wall to permit efficient distribution of water within their aerial tissues [23].

Uronic acids and low-buffering capacity of the environment are responsible for the acidic character of *Sphagnum* moss. Uronic acids become ionized at pH above 2. Phenolics on the contrary ionize at alkaline pH and therefore do not contribute to ion-exchange at pH < 7 [24]. Uronic acids in *Sphagnum* create new cation exchange sites as the *Sphagnum* grows which leads to continuous exchange of hydrogen ions for cations in mire water [8, 24].

In 1983, Terrence J Painter isolated an unusual oxopolysaccharide from *Sphagnum* leaves' cell walls and named it "sphagnan" [25]. Later on, he suggested that sphagnan has unique functional properties that distinguish it from other plant polysaccharides [26]. Sphagnan is structurally related to the "complex pectins" of higher plants but while containing approximately 25 % of ketouronic acid with highly reactive carbonyl groups (also named as α -keto-carboxylic acids); it differs from other plant sugars.

Table 3 Concentrations of main components in peats of different degrees of decomposition [104]

Component	Percent of dry weight [%]		
	Not decomposed (H ₁₋₂)	Moderately decomposed (H ₅₋₆)	Completely decomposed (H ₉₋₁₀)
Cellulose	15–20	5–15	–
Hemicellulose	15–30	10–25	0–2
Lignin-like substances	5–40	5–30	5–20
Humic substances	0–5	20–30	50–60
Bitumen	1–10	5–15	5–20
Nitrogenous substances	3–14	5–20	5–25

Classification H1–H10 was based on the Von Post scale

2.2 Phenolics

Phenolics, i.e., polyphenols, are a wide group of secondary metabolites that function in various roles, such as in plant defense. Phenolics constitutes of a large variety of molecules that can be grouped based on their precursor. L-phenylalanine derivatives include phenylpropanoids, condensed tannins, lignin, flavonoids, and hydroxycinnamic acids whereas gallic acid derivatives include hydrolysable tannins [27].

Bryophytes contain a relatively low amount of phenolics, although they are more abundant in *Sphagnum* mosses than in other bryophytes [21]. *Sphagnum* phenolics were historically referred to as sphagnol [8]. At the present, this term is not preferred since it would represent a mixture of different phenolics. The composition of phenolics in *Sphagnum* peat depends on the degree of decomposition; the amount of biologically active phenol compounds flavonols seems to correlate with the degree of decomposition in peat [28] (Table 3).

Analyses adopted from wood chemistry have shown lignin-like phenolics in *Sphagnum* (e.g., presented as Klason lignin content) [29, 30]. However, structural analyses have not supported their existence [21]. The cell wall surface of *Sphagnum* mosses is coated with amorphous phenolic polymers that protect amorphous components of the *Sphagnum* cell wall such as mono- and disaccharides, cellulose, and pectin. Phenolics form a polyphenol network that provides both chemical and physical barrier against degrading microorganisms [31].

Sphagnum synthesizes specific polyphenolic compounds such as *Sphagnum* acid and reddish-violet pigments sphagnorubins. Sphagnorubins are responsible for the color change of *Sphagnum* from green to red during a vegetation period in response to cold nightly temperatures. Structurally, they are flavonoid derivatives with a phenyl-phenatro-pyran ring skeleton [32].

Sphagnum acid (p-hydroxy- β -carboxymethyl-cinnamic acid) is a decomposition product of lignin-like phenolics, synthesized via the Shikimate pathway [33]. It is the most abundant phenolic compound in *Sphagnum* [33, 34]. Williams et al. detected also the following phenolics in *Sphagnum*: p-hydroxyacetophenone, p-hydroxybenzoic acid, SA [p-hydroxy- β -(carboxymethyl)-cinnamic acid], p-hydroxybenzaldehyde, vanillin (4-hydroxy-3-methoxybenzaldehyde), p-coumaric acid (4-hydroxycinnamic acid), acetovanillone (4-hydroxy-3-methoxyacetophenone), syringaldehyde (4-hydroxy-3,5-dimethoxybenzaldehyde), vanillic acid (4-hydroxy-3-methoxybenzoic acid), ferulic acid (4-hydroxy-3-methoxycinnamic acid), and syringic acid (4-hydroxy-3,5-dimethoxybenzoic acid) [34]. Morita reported isolation of a rarely occurring p-hydroxyacetophenone ester of palmitic acid from a *Sphagnum* peat [35].

3 *Sphagnum* properties and respective applications

The most famous properties of *Sphagnum* are related to preservation function, antimicrobial activity, and high adsorption capacity. Although molecular origins of these properties are not fully understood, they have been used for centuries in food preservation and medicine. During the recent decades, *Sphagnum* biomass has also been utilized for a wide variety of other applications (Table 4). The current knowledge on *Sphagnum* properties and their mechanisms together with tissue or extract level applications are discussed in the following chapters.

3.1 Preservative properties

In August 1984, the so-called Lindow man was found in a *Sphagnum*-dominated peat bog in northern Cheshire, UK. The body was 2000 year old and still well-preserved. The skin and other collagenous tissues of the body were tanned like leather [36]. Until ca. 1990, the preservative effect of peat was examined via such preserved human bodies. The major observations were that anoxic conditions occur approximately 40 cm from the surface peat lands and below, and that the remained bodies are usually almost completely decalcified with the skin and other collagenous tissues colored dark brown and tanned. The preservation is enhanced due to the enormous adsorption of water that basically blocks heat transfer within the peat and thus the deeper regions of peat lands remain cool even in summer [37]. In nature, some birds use moss in order to protect their nests against biodegrading microbes [38]. The moss used in nests could for example protect birds against *Bacillus licheniformis* [39].

3.1.1 On the origin of preservative properties

Various explanations have been offered for the preservative nature of peat, yet the actual mechanism behind *Sphagnum*'s antimicrobial activity remains still disputed [31, 40]. A general opinion among ecologists has been that the anoxic

conditions are partly responsible for the preservation of peat and of objects preserved in peat. Basically, there are two different suggestions to explain the resistance of *Sphagnum* against decay, polysaccharides and phenolic compounds. Nevertheless, the influence of these compounds on microorganisms is not clear because in nature there are always many factors affecting the process, such as low temperature, acidity, lack of oxygen, and lack of nitrogen and other nutrients [14].

Sphagnum mosses possess both passive and active mechanisms against mineralization. The supposed existence of passive mechanisms is based on observed relation between the proportion of metabolic and structural carbohydrates in *Sphagnum* and the rate of litter mineralization [5]. Active mechanisms are assumed to include slow release of pectin-like polymers, such as rhamnogalacturonan I, into the environment during the hydrolysis of cell walls [25, 41, 42]. These molecules, referred to as sphagnan, lower the pH of the environment and may also form polyelectrolyte complexes with free amino groups of extracellular enzymes, thus inactivating them [43]. Such reactions lead to carbon and nitrogen limitation and thus also indirectly inhibit microbial growth. Although suggested effect of carbonyl groups in sphagnan has been later disproved [37, 43, 44], it is evident that cell wall polysaccharides are important factors in the slow mineralization of *Sphagnum* litter [30, 45]. Interspecific differences in decay rate within *Sphagnum* may relate to amounts of easily and poorly degradable polysaccharides within the moss [5, 30].

In their earlier research, Painter et al. suggested that galacturonic and 5-keto-D-mannuronic acid (5-KMA) in sphagnan [26] contributed to the preservative effect by binding nitrogen (N) and that way making it unavailable for microorganisms [37]. However, this theory was recently revised within the same research group proposing that 5-KMA was inexistent in *Sphagnum papillosum* and perhaps also in other *Sphagnum* species [46]. The present understanding is that the carboxyl group of galacturonic acid (GalA) in pectin does possess microbicidal properties in the form of proton release into solution in exchange for cations. This mechanism is respective to pH

Table 4 Properties *Sphagnum* moss and respective applications

Property	Raw material	Suggested applications
Preservative properties	Plant tissues, holocellulose	Food packages [37], preservation of archeological findings [57]
High adsorption capacity	Plant tissues	Food packages and adsorption pads [55], wound bandages and surgical dressings [62, 64], peat-cellulose fabric as oil collection cloth [66], carbon nanomaterials [65], biosorbent of metals [100]
Cation exchange capacity	Plant tissues, holocellulose [41]	Water purification [101, 105, 106]
Antioxidative function	Plant extract [107]	Functional foods [68], medicine [70]
Antimicrobial properties	Plant extracts	Use of tissues or extracts in medicine [53] [86] [70], medicinal materials [108], or cosmetics

[29]. Sphagnum may also inhibit microbial growth via electrostatic immobilization of extracellular enzymes [30, 43].

Sphagnum is a weak organic acid [42] which means that under low pH it takes its uncharged, undissociated form and is able to permeate across the plasma membrane and thus enter the bacterial cell. When the molecule reaches neutral cell cytoplasm, it dissociates which results in the accumulation of anions and protons inside the cell [47]. As a weak acid, sphagnum also affects microbial growth by decreasing the pH of environment although with rather low-buffering capacity [42].

It was also suggested that sphagnum would not be cytotoxic but would instead cause nitrogen deprivation for microbes [48]. This is supported by reports of intense leucocyte proliferation at the interface between wounds and *Sphagnum* dressings. A supposed mechanism could be nitrogen removal via Schiff base formation (C=N) with certain amines including ammonia, proteins, and enzymes [37]. When ammonia or primary amines are present, the reaction goes fast and nitrogen becomes irreversibly incorporated into the structure of the brown chromophore. This reaction is a Maillard reaction which results to the dark brown polymeric end-product melanoidin [26, 49].

Regarding the sphagnum's close relationship to pectic acid, it is plausible that it should bind calcium and other multivalent metal cations strongly. This property seems to be rather a merit of polyanionic character than content of reactive carbonyl groups [37]. Calcium-binding effect has been confirmed for both soluble sphagnum and its insoluble, wall-bound form in *Sphagnum* [50]. The growth of calcium ion-dependent bacterium *Azotobacter vinelandii* is strongly inhibited by sphagnum. Similar effect has been observed when natural humic acid isolated from peat bog water or artificial humic acid prepared by heating aqueous sphagnum were used [25]. Accordingly, such components of peat which sequester multivalent cations are partly responsible for the low microbial activity in peat.

Although evidence on actual antimicrobial properties of *Sphagnum* phenolics is still lacking, correlations between phenolics and mineralization rate of *Sphagnum* litter have been found. It is still debatable whether the effect is due to phenolics excreted by *Sphagnum* or those originating from decomposition of lignin-like phenolic polymers. However, it is possible that phenolics are involved in the formation of humic substances and sequestration of organic nitrogen and phosphorous. Phenolics are also assumed to inhibit the activity of extracellular enzymes which may contribute to the antimicrobial effect of *Sphagnum*.

3.1.2 Applications based on preservative properties

The preservative properties of *Sphagnum* moss have traditional applications in Nordic countries. *Sphagnum* has been used as packing material to store foods such as freshly caught fish

and root vegetables [42]. Tar extracted from peat moss was historically used as a weak antiseptic for the external treatment of eczema and other skin diseases [51, 52].

Bryophyte extracts may generally be more effective against gram-positive than gram-negative bacteria, which is perhaps due to physiological differences [53]. In a study of various Indian mosses [54], *Sphagnum junghuhnianum* ethanol extract was shown to have an antibacterial effect against *Micrococcus luteus*, *Bacillus subtilis*, *Bacillus cereus*, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhimurium*, and *Streptococcus pneumoniae*. Antimycotic activity was detected against *Candida albicans*, *Cryptococcus albidus*, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus terreus*, *Aspergillus nidulans*, *Aspergillus spinulosus*, and *Trichophyton rubrum*. However, in another study, alcohol extracts of *Sphagnum palustre* showed no antibiotic effect against *E. coli*, *K. pneumoniae*, or *B. subtilis* [53]. In case of some other bryophytes, the extraction solvent influenced on antibiotic activity, ethanol extracts being more active against tested bacteria than methanol extracts. Thus, the author concluded that compounds that are extracted to ethanol but not to methanol, such as sterols or polyacetylenes, are responsible for the antibiotic effect.

The preservation capacity of *Sphagnum* for fish has been studied by Borsheim et al. They examined the storage effects to strips of salmon skin or whole zebra fish by embedment either in untreated *S. palustre* moss, peat derived mainly from *Sphagnum* mosses, acetone-extracted moss, or the chlorite holocellulose of the moss [37]. Preservation took place for several weeks in the presence of oxygen at 20–23 °C and pH 3.4–5.1. The chlorite holocellulose of the moss was pure, insoluble, white polysaccharide, and it acted as well or better than other materials. It contained approximately 0.5 mmol/g highly reactive carbonyl groups. When the carbonyl groups were removed by borohydride reduction or condensation with ammonia, the preservative property was lost. At the same time with preservation, browning of the dermis and a yellowing of the scales occurred. These phenomena were supposedly related to the Maillard reaction, because neither of them occurred after the carbonyl groups were removed.

In further examination, a water-soluble fragment was extracted from the chlorite holocellulose and analyzed to contain 1.0 mmol/g of carbonyl groups. This fraction, referred to as sphagnum, produced the same kind of effects as embedment in any of the examined materials, even though more rapidly. The observations revealed that coloring and bio-resistance of fish took place after immersion in aqueous sphagnum while the borohydride-reduced Sphagnum did not promote preservation. Still, it has been concluded that filleted fish cannot be preserved by this way due to the neutralizing of carbonyl groups by soluble proteins that leak out from the muscle.

Stalheim et al. proved that sphagnum in its acid form is able to inhibit growth of various food poisoning and spoilage bacteria on low-buffering solid growth medium [42]. The effect is, however, limited to acid-sensitive species such as *B. cereus*, *S. aureus*, *Listeria monocytogenes*, *Pseudomonas* spp., *Aeromonas* spp., and *Shewanella* spp. Four of five strains of *Salmonella* spp. were able to grow even in wells with a concentration of 5 mg/mL of protonated sphagnum while all five tested strains of *E. coli* were inhibited by 4 mg/mL of protonated sphagnum. These concentrations correspond to pH of 3.9 and 4.1, respectively. Prior autoclaving or sterile-filtration did not affect the inhibition ability of sphagnum. In its sodium form, sphagnum could not inhibit the bacterial growth of the tested strains in the low-buffering medium. The antibacterial activity of sphagnum was comparable to that of hydrochloric acid or rhamnogalacturonan pectin in its acidic form. In its sodium form at neutral pH, sphagnum had no antibacterial activity.

As sphagnum in its acid form is a weak macromolecular acid, it could be used for example as a food preservative and additive like other weak organic acids (such as acetic, lactic, benzoic, and sorbic acids). It is known that these organic acids can inhibit the growth of both bacterial and fungal cells [47]. Such use, however, would be perhaps limited due to availability of many cheaper alternatives already on the market [42].

Absorbent pads made from bleached *Sphagnum* were shown to extend the shelf-life and improve the quality of packed salmon fillet slices [55]. Ballance and Christensen have patented a moss-derived antimicrobial agent, suitable for, e.g., treatment of surfaces in the food industry [56]. Nowadays, freshly harvested *Sphagnum* moss is the recommended packing material for transportation of excavated objects in Canadian Arctic archeology. Objects packed in *Sphagnum* moss both keep their natural humidity and are protected against microbial colonization [57, 58]. It has been suggested that *Sphagnum* might also preserve wool by binding with keratins [59].

3.2 Adsorption capacity

Sphagnum hyaline cells can hold 16–25 times their dry weight of water [60]. Water can run up to 30 cm from water bed through hyaline cells due to osmotic pressure [61]. At water potentials less than -10 kPa, the majority of water in *Sphagnum* is held by hyaline cells. At higher water potentials, more water is held in external capillary spaces between the leaves [4]. Due to these water transport mechanisms, *Sphagnum* moss is a very effective absorbent [62]. It can hold large quantities of water, even 20 times its dry weight [52, 63]. This property has been utilized in dress wounds, e.g., during the World Wars to substitute cotton [64]. The absorption ability of *Sphagnum* was reported to be 3–4 times higher than that of cotton equivalents [48].

A recent report described the adsorption capacity of *Sphagnum fuscum*-based carbon nanotubes [65]. *Sphagnum* was processed by drying and milling to 100–150 mm particle size. Carbon with an amorphous structure was obtained at 950 °C after which it was eluted from minerals using a mixture of nitric acid and hydrochloric acid at 100 °C. Thereafter, the amorphous carbon was separated from the mixture of the acid solutions on by filtration and water washing, and dewatered by centrifugation and heating. Amorphous carbon was further processed using mechanochemical milling. The sorption capacity values for the carbon nanotubes were order of magnitude higher than the values recorded for the studied types of activated carbons. The sorption capacity and specific surface of the tubes diminished with prolongation of storage.

A biodegradable peat-cellulose fabric was prepared by pressing *Sphagnum* peat between two wet layers of cellulose, and by adding polyester fibers to the top cellulose layer for improved strength [66]. The oil absorption capacity of the peat-containing fabric was comparable to commercial products. In another study, it was found that addition of small amounts of peat humic acids to hydrogel decreased the viscosity and increased the spreading coefficient of adhesive over the cellulose surface [67].

3.3 Sphagnum in human nutrition, medicine, and cosmetics

Antioxidant can be defined as a molecule, which significantly delays or inhibits the oxidation of other molecules. Antioxidants prevent the action of free radicals that are involved in the development of, e.g., cardiovascular or neurodegenerative disorders. Accordingly, consumption of antioxidant-rich plants have shown to prevent various diseases [68]. Approximately 10–30 % of the dry mass of *Sphagnum* comprises of uronic acids which are known to possess antioxidative properties [69]. The antioxidant capacity of *Sphagnum magellanicum* has been determined for dietary supplement purposes. The capacity was equivalent in ORAC (oxygen radical absorbance capacity assay) units to 841 $\mu\text{mol Trolox}/100$ g units [70] which is promising regarding possible use as a functional food [71]. In a study by [72], differences in antioxidant activity were observed between peat varieties.

The nutritional properties of *Sphagnum* were investigated by Villarroel et al. [73]. Chemical characteristics of *S. magellanicum* were shown suitable for the purpose: the amount of dietary fiber was 77 % which is higher than in, e.g., rice hull, barley hull, or oat bran, and no factors related to antinutritional effects were detected [74]. Water absorption and water retention capacities, swelling capacity, organic molecule absorption capacity, and cationic interchange capacity were analyzed for several fiber fractions that were also applied

as fiber source in bakery products [73]. *S. magellanicum* was also used among other fiber sources in formulation of a functional pastry product [75].

Sphagnum mosses contain complex mixtures of lipids, e.g., C28C29 sterols, C-30 triterpenoids, C-16-C-30 fatty acids, C-22-C-30 fatty alcohols, C-21-C-33 n-alkanes, and isoprenoid and straight-chain wax esters [76]. Triterpenoid concentrations vary between *Sphagnum* species. Reported summed amounts have been between 20 and 3500 $\mu\text{g/g}$ dry weight. Small concentrations were present in the mesotrophic species *Sphagnum fimbriatum* and *S. palustre*. The major triterpenoid detected was ursolic acid, which is suggested to be effective in protecting against various disorders, including induced liver injury [77]. Ursolic acid possesses a wide range of biological functions, such as antioxidative, anti-inflammation, and anti-cancer activities (although associated also with some pro-inflammatory functions) [78, 79], and it has been in human clinical trial for treating cancer, tumor, and skin wrinkles already for several years [80].

At least seven pure flavonoids have been isolated from *Sphagnum*, including flavones apigenin, apigenin-7-O-triglycoside, lucenin-2, luteolin-7-O-neohesperidoside, saponarine and vitexin, and the biflavonoid bartramiaflavone. These may open up applications in nutrition or medicine. Many flavonoids alter metabolic processes having a positive impact on health and thus being an attractive supplement in functional foods. There are also various medicinally interesting compounds within the flavonoids isolated from *Sphagnum*.

Apigenin (4', 5, 7-trihydroxyflavone) has been shown to possess remarkable anti-inflammatory, antioxidant, and anti-carcinogenic properties [81]. Vitexin has shown potential for the prevention of UV-induced adverse skin reactions such as free radical production and skin cell damage [82]. Based on studies using rat cells, fulvic acids of *Sphagnum* peat may also have anti-allergenic properties [83]. Humic acids and poly(OH)carboxylates present in *Sphagnum* peat are also selective inhibitors of Herpes simplex virus [84]. Furthermore, oxihumate—a water-soluble compound of peat—has been shown to inhibit HIV-1 infection of MT-2 cells.

Flavonoids of *Sphagnum* were shown to have pronounced antibacterial effects against *Enterobacter cloacae*, *E. aerogenes*, and *P. aeruginosa* [85]. Phenolics of *Sphagnum* may also have inhibitory effect against certain round worms [86]. The effect of milled *Sphagnum* leaves on bacterial growth may be significantly higher than that of isolated compounds which suggest that the extraction of phenolics for such use is not feasible [87].

3.4 *Sphagnum* as substrate in bioprocesses

Sphagnum peat hydrolyzates have been utilized in various fermentation processes, for production of biomass and/or

specific microbial products (Table 5). Regarding the typical carbohydrate, amino acid, and mineral concentrations, *Sphagnum* and its peat thereof are well suitable for fermentation processes. Dried *S. papillosum* was reported to contain 37.39 % of hexose sugars [29]. It is however notable that the polysaccharide composition of peat depends on its degree of decomposition (Table 3) which should be taken into account when designing the hydrolysis process.

Peat extracts have been studied for production of edible mushrooms *Agaricus campestris*, *Morchella esculenta*, and *Pleurotus ostreatus*. These were grown using peat extracts as the primary substrate [88–90]. Fungi have also been cultivated on peat hydrolyzates for production of single-cell protein in several cases. Cultivation of an acidophilic fungus in an open pond bioreactor resulted to maximum biomass concentration of 10.4 g/L and the total carbohydrate reduction of 69.7 % [91]. This was achieved using medium composition of 0.5 % $(\text{NH}_4)_2\text{SO}_4$, 0.5 % K_2HPO_4 , 0.04 % MgSO_4 , and an initial pH of 2.5. The fungal biomass contained 24.3 % protein, 1.7 % lipid, 61.2 % carbohydrate, and 12.8 % ash (per dry weight), and its composition of essential amino acids was clearly above the food standards.

The yeast *Rhodotorula rubra* was cultivated on acid extracts of peat for carotenoid production. At optimal growth approximately 4.8 g/L (dry weight) biomass was produced with β -carotene concentration of 1256 $\mu\text{g/g}$ dry biomass [92]. Another yeast-synthesizing carotenoid, *Phaffia rhodozyma*, was cultivated for the production of astaxanthin. The mean astaxanthin content of the yeast was 1567 $\mu\text{g/g}$ dry yeast. Peat hydrolyzate was also used for production of surfactin in *B. subtilis* in quality that was comparable to production in conventional glucose and mineral salts medium [93].

A diluted acid-autoclaved extract of peat was used as a substrate for the production of the extracellular polysaccharide pullulan by *Aureobasidium pullulans* [94, 95]. The hydrolysis was carried out in 1.5 % (v/v) sulfuric acid at 140 °C for 2 h. The performance of peat hydrolyzate in pullulan production was similar to glucose medium. Suggested economically optimized culture medium for large-scale production of pullulan contained peat hydrolyzate, 0.05 % NaCl, 0.02 % MgSO_4 , and 0.01 % antifoam FG-10. The used peat was an effluent of the peat processing industry. The process was later on further optimized via feeding strategies based on examination of fermentation kinetics [96]. In a more recent study, acid peat hydrolyzate was used for cultivation of *A. pullulans* to produce pullulan and simultaneously accumulate metals from the medium [97]. Up to 97.3 % of Fe and 62.05 % of Zn could be removed. The concentrations of Cd, Ni, and Cr could be reduced at best by 35–52 %. The metals were accumulated in the biomass and not bound to the pullulan.

Table 5 Use of *Sphagnum* moss or peat as substrate in bioprocesses

Substrate	Microbe	Product	Reference
Peat hydrolyzate	<i>Phaffia rhodozyma</i>	Astaxanthin	[109]
Peat extract	<i>Rhodotorula rubra</i>	Carotenoid-rich biomass	[109]
Dried moss	<i>Aspergillus niger</i>	Citric acid	[110–113]
Peat hydrolyzate	<i>Agaricus campestris</i> , <i>Morchella esculenta</i> , <i>Pleurotus ostreatus</i>	Edible mushrooms/mycelia	[88–90, 114–116]
Fish offal peat compost	<i>Candida utilis</i>	Single-cell protein for feed	[117, 118]
Moss hydrolyzate	<i>Scytalidium acidophilum</i>	Single-cell protein for feed	[119]
Peat hydrolyzate and fish waste	Lactic acid bacteria	Silage	[120]
Peat hydrolyzate	<i>Bacillus subtilis</i>	Surfactin	[93]
Peat hydrolyzate	<i>Aureobasidium pullulans</i>	Pullulan ^a	[94–96]

^a Pullulan production has been combined with metal biosorption in [97]

4 Fractionation and isolation of compounds of interest

Sphagnum mosses' commercially attractive properties and respective tissue level applications were discussed in a previous chapter. The systematic use of this abundant biomass would, however, benefit from component level investigation of potential processing options. Furthermore, well scalable extraction processes, such as hot water extraction or ethanol extraction, have been successfully used for the isolation of various valuable compounds of moss (Table 6).

Before chemical processing, *Sphagnum* leaves are usually dried in oven or under air current for several days. Milling or grinding may be used to ensure homogeneity [46, 87, 98].

Holocellulose of *Sphagnum* leaves may be extracted as described by Ballance et al. [46]. The preparation begins with the removal of waxes and pigments from the leaves by means of acetone extraction. The leaves are boiled in acetone at 57 °C for 3 min and filtrated through a nylon filter mesh (pore size 60 µm). In the reviewed study, 40 mL of acetone was used for extraction of 1 g of leaves. Extraction and filtration should be repeated several times to achieve a nearly colorless filtrate. The leaf residue is then extracted once more with dry methanol and air-dried in the fume hood at room temperature. Acidified sodium chlorite can be used to selectively oxidize

aromatic compounds in *Sphagnum* leaves while their polysaccharide structure remains largely intact [99]. After this treatment, the leaves are filtered, washed well with water followed by ice-cold 0.02 N HCl, and finally washed again with distilled water until the washings are neutral. To give leaves their protonated form, the residue is washed with acetone and methanol, and air-dried. Various applications have been suggested for *Sphagnum* holocellulose, e.g., related to water purification [66, 100, 101]. It is however a source of various commercially interesting compounds suggesting that it should rather be regarded as an intermediate rather than the end production of the fractionation process.

Sphagnan is located within the cell walls of *Sphagnum*. It is also the source of uronic acids which comprise 10–30 % dry weight of *Sphagnum* [4]. For preparation of sphagnan, a suggested protocol includes drying of freshly collected leaves under a current of air of 60 °C, followed by previously described holocellulose preparation, and finally isolation of sphagnan. Sphagnan can be prepared from holocellulose extract as follows. Dry phenol-free leaves are made into a thick slurry with 2 l degassed distilled water which is then heated at 98 °C. The residual solids are collected by vacuum filtration. The solids are re-suspended in distilled water and then heated again. When the slurry is too thick to filter in a reasonable time, centrifugation is used for the collection of precipitate.

Table 6 Commercially interesting components of *Sphagnum* moss

Component	Isolation steps	Suggested applications
Holocellulose	Removal of extractives and bleaching [46, 121]	Cation exchanger material in water purification [101]
Sphagnan	Hot water extraction of holocellulose fraction [42, 121].	Antimicrobial additive in coating materials [122]
Sugars	Acid hydrolysis of holocellulose	See Table 4
Triterpenoids	Chloroform-ethanol extraction [123], ethanol extraction [103], acetone extraction [43]	Medicine [77, 78, 124], cosmetics [80]
Flavonoids	Ethanol extraction [28]	Bioactive compounds in foods [68, 85] and medicine [85, 125]

The supernatant is decanted off and pooled with the earlier filtrates. Filtrates are further concentrated, e.g., in a rotary evaporator at low temperature and filtrated through 0.45 and 0.22 μm membranes (Millipore nitrocellulose). The sphagnum can be converted to Na^+ -form by means of dialysis against NaCl and water. To prevent microbial spoiling, the extract may be sterile filtered. This procedure has yielded 10 g or sphagnum from 33 g of holocellulose [46].

Acid hydrolysis is well suitable for the saccharification of cellulose and hemicellulose of *Sphagnum*. Sulfuric acid has been used for the purpose in various studies. The reported conditions are in the range of 121–140 $^{\circ}\text{C}$ and 0.5–2 % sulfuric acid. A maximum sugar concentration of 7.7 g/l was obtained with glucose accounting for 46 % of the total [93]. It is notable that mechanochemical treatment may change the composition of polysaccharides in *Sphagnum*.

Phenolics can be isolated from *Sphagnum* leaves according to chlorite treatment described for the sphagnum preparation. This protocol yields 60 $\mu\text{g/g}$ p-hydroxyacetophenone, 25 $\mu\text{g/g}$ p-hydroxybenzoic acid, and 20 $\mu\text{g/g}$ *Sphagnum* acid together with minor quantities of p-hydroxybenzaldehyde, vanillin, p-coumaric acid, acetovanillone, and syringaldehyde [87]. Phenolics may also be extracted by means of ethanol extraction, yielding approximately 5 mg of phenolics from a gram of *Sphagnum* peat [98].

Lipid extraction from *Sphagnum* has this far targeted to compositional study of the moss. Baas et al. used methanol/dichloromethane extraction for comprehensive isolation of *Sphagnum* lipids. Maximum yield of triterpenoids was 3.52 mg/g of moss (isolated from *Sphagnum compactum*) from which half is comprised of ursolic acid. Jansen et al. used accelerated solvent extraction (ASE) for the extraction of lipid biomarkers of *Sphagnum* [102]. The feasibility of these methods in a commercial scale was not discussed. Examples regarding other plant species suggest that ethanol would be an efficient solvent for the isolation of triterpenoids, including ursolic acid, from *Sphagnum*. Chen et al. compared various solvents for the isolation of saponins from *Ganoderma atrum* fungus [103]. Highest yield of triterpenoids was achieved by microwave-assisted ethanol extraction. This, however, will also yield other components, and will thus not serve in selective fractionation. Acetone extraction would perhaps serve better for the purpose [46] although it would require subsequent removal of pigments.

5 Conclusions

The reviewed literature suggests that *Sphagnum* is a potential raw material for various high-valued commodities. Although molecule or macromolecule level use of *Sphagnum* is still rarely pursued, mostly due to lack of cost-efficient isolation procedures, the interest towards valuable organic monomeric

molecules present in *Sphagnum* is likely to emerge in the future. Especially, terpenoids and uronic acids seem to have potential for high-valued applications, such as pharmaceuticals, cosmetics, and biodegradable materials.

Another attractive alternative would be the combination of extraction and recovery of monosaccharides with subsequent fermentative conversion of sugars to microbial cell mass or metabolites. Up to date, the presented bioprocesses are largely based on *Sphagnum* peat rather than the mother plant. However, regarding the different compositions of moss and decomposed peats, *Sphagnum* moss could actually be a more suitable material for hydrolysis than peat and would also provide more hexose sugars to fermentation processes.

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