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Research review paper

## Using agro-industrial wastes for the cultivation of microalgae and duckweeds: Contamination risks and biomass safety concerns

Giorgos Markou<sup>a,\*</sup>, Liang Wang<sup>b</sup>, Jianfeng Ye<sup>b</sup>, Adrian Unc<sup>c</sup><sup>a</sup> Institute of Technology of Agricultural Products, Hellenic Agricultural Organization – Demeter, Leof. Sofokli Venizelou 1, Likovrysi 141 23, Greece<sup>b</sup> Water Research Institute, Shanghai Academy of Environmental Sciences, 508 Qinzhou Rd, Shanghai 200233, China<sup>c</sup> School of Science and the Environment, Memorial University of Newfoundland, 20 University Drive, Corner Brook, NL A2H 5G4, Canada

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## ABSTRACT

Aquatic organisms, such as microalgae (*Chlorella*, *Arthrospira* (Spirulina), *Tetraselmis*, *Dunaliella* etc.) and duckweed (*Lemna* spp., *Wolffia* spp. etc.) are a potential source for the production of protein-rich biomass and for numerous other high-value compounds (fatty acids, pigments, vitamins etc.). Their cultivation using agro-industrial wastes and wastewater (WaW) is of particular interest in the context of a circular economy, not only for recycling valuable nutrients but also for reducing the requirements for fresh water for the production of biomass. Recovery and recycling of nutrients is an unavoidable long-term approach for securing future food and feed production. Agro-industrial WaW are rich in nutrients and have been widely considered as a potential nutrient source for the cultivation of microalgae/duckweed. However, they commonly contain various hazardous contaminants, which could potentially taint the produced biomass, raising various concerns about the safety of their consumption. Herein, an overview of the most important contaminants, including heavy metals and metalloids, pathogens (bacteria, viruses, parasites etc.), and xenobiotics (hormones, antibiotics, parasiticides etc.) is given. It is concluded that pretreatment and processing of WaW is a requisite step for the removal of several contaminants. Among the various technologies, anaerobic digestion (AD) is widely used in practice and offers a technologically mature approach for WaW treatment. During AD, various organic and biological contaminants are significantly removed. Further removal of contaminants could be achieved by post-treatment and processing of digestates (solid/liquid separation, dilution etc.) to further decrease the concentration of contaminants. Moreover, during cultivation an additional removal may occur through various mechanisms, such as precipitation, degradation, and biotransformation. Since many jurisdictions regulate the presence of various contaminants in feed or food setting strict safety monitoring processes, it would be of particular interest to initiate a multi-disciplinary discussion whether agro-industrial WaW ought to be used to cultivate microalgae/duckweed for feed or food production and identify most feasible options for doing this safely. Based on the current body of knowledge it is estimated that AD and post-treatment of WaW can lower significantly the risks associated with heavy metals and pathogens, but it is yet unclear to what extent this is the case for certain persistent xenobiotics.

### 1. Introduction

More than 15% of the people today do not have access to sufficient protein and energy in their diets, and even more suffer from micronutrient malnutrition (Godfray et al., 2010). The increasing trend of population growth (United Nations, 2017), the intensification of industrialization and depletion of resources, the inefficient utilization of nutrients for food and feed production, all in the context of the observed and projected climate changes, will expectedly accentuate the impacts on the environment. Furthermore, the decline of available irrigation water and arable land, along with rising global temperatures

that destabilize farming systems, possess a threat for feed and food security (Godfray et al., 2010; Vermeulen et al., 2012; Wheeler and von Braun, 2013). All these pressures make the searching for alternative ways for producing feed and food, unavoidable.

Today feed and food production is based mainly on the cultivation of terrestrial plants. It is expected that protein consumption will increase, resulting in a higher demand for protein rich feed. In the EU most vegetable feed for animal consumption is based on soybean (35–40% proteins) which, given that its growth requirements are met most easily in sub- and tropical climates and require large land areas, is imported from third countries mainly Brazil and USA. However, the

\* Corresponding author.

E-mail address: [markoug@aua.gr](mailto:markoug@aua.gr) (G. Markou).<https://doi.org/10.1016/j.biotechadv.2018.04.003>

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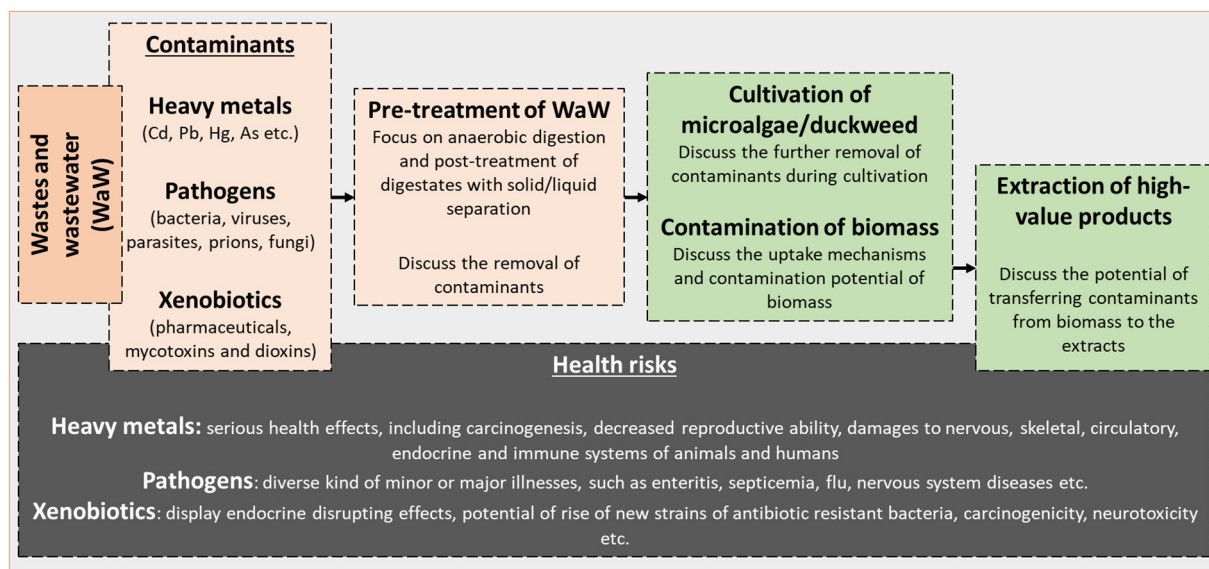


Fig. 1. Schematic overview of the main discussion points of cultivation of microalgae/duckweed using agro-industrial wastes and wastewater.

sustainability of soybean production on lands converted from forest or pasture use (Arima et al., 2011; Fehlenberg et al., 2017) and of its long-distance importation is questionable, making the long-term stability of any locally or globally feasible food-security programme dependent on finding alternative ways of producing protein-rich biomass for feed or food production (Taelman et al., 2015). Although improvements in crop yields through improved cultivation and production practices can be projected (Balafoutis et al., 2017; Vermeulen et al., 2012), alternative feed and food production systems must be considered for true food security (Rumpold and Schlüter, 2013; Walsh et al., 2015).

Microalgae (including cyanobacteria) and aquatic plants such as duckweed (for example *Lemna* spp. or *Wolffia* spp.) are a very promising renewable source of nutrients and high-value compounds that could be used as feedstock for the production of a wide range of products (Appenroth et al., 2017; Borowitzka, 2013; Christaki et al., 2011; Laurens et al., 2017; Spolaore et al., 2006). Besides proteins, they also contain fatty acids, pigments, anti-oxidants etc., that are a very interesting source to be used as food and feed supplements (Christaki et al., 2011; Leng, 1999; Markou and Nerantzis, 2013; Pulz and Gross, 2004). Some microalgal species, such as *Arthrospira* (Spirulina), *Chlorella*, and *Dunaliella* are rich in proteins (> 50%<sub>dw</sub>), display good productivities (20–30  $t_{DM} ha^{-1} year^{-1}$  (Walsh et al., 2015)) and have a favorable amino acid profile compared to that of reference and food proteins (Becker, 2007). Given that cultivation of microalgae and duckweed offers some interesting options, such as a year-round biomass production and continuous harvesting, use of non-arable land, and use of brackish- or sea-water as the basis of the cultivation medium, they have been long suggested as a potential protein source to cope with increasing global food demand (Rusoff et al., 1980; Spoehr, 1953).

For a sustainable food and feed production, recovery and recycling of nutrients that are lost with waste and wastewater is of particular importance. Nutrients recovery and recycling is an unavoidable long-term approach for securing food and feed production (Schoumans et al., 2015; Zacharof and Lovitt, 2015). Microalgae and duckweed have been proven to integrate highly efficient nutrient recovery from wastewaters while producing highly versatile biomass (Cheng and Stomp, 2009; Olguín, 2012; Solovchenko et al., 2016). Potential waste and wastewater streams that have been investigated for nutrient recovery include municipal wastewater, agro-industrial WaW, such as poultry, piggery, cattle, food wastes and food processing by-products (cheese whey, olive-oil mill wastes etc.), as well gaseous waste streams such as flue gases. The aptitude of microalgae and duckweed to grow on a range of

waste streams has been extensively reviewed (Abinandan and Shanthakumar, 2015; Cai et al., 2013; de la Noüe and de Pauw, 1988; Iqbal, 1999; Journey et al., 1991; Markou and Georgakakis, 2011; Markou et al., 2014; Van Den Hende et al., 2012, 2016; Whitton et al., 2015).

Nevertheless, waste streams, besides their content in valuable nutrients, may contain also various other organic and/or inorganic compounds that could contaminate the biomass, rendering it unsuitable for feed or food production. Such contaminants include inorganic pollutants like heavy metals & metalloids (HMs), pathogens, and various organic pollutants. The lack of a comprehensive review on this topic prompted us to try filling this gap. Due to the high heterogeneity in the physico-chemical characteristics of WaW, the paper will focus only on the nutrient rich agro-industrial waste streams, especially on manures and livestock wastewater. A particular focus is put on waste streams from AD technology, which is widely used to treat the agro-industrial WaW; the digestion effluents (digestates) preserve most of the inorganic nutrients from the feedstock and therefore they have been suggested as a promising source of nutrient source for microalgae and duckweed cultivation (Markou, 2015; Wang et al., 2010). We discuss the presence of pollutants that have potential health risks, the mechanism of their uptake by the cells, and pre-treatment methods that might possibly decrease the contamination of the biomass. In the following sections the most important potential contaminants from the agro-industrial WaW streams that could influence the quality of the produced biomass are discussed. The main categories of contaminants that are taken into consideration are: HMs, pathogens, and xenobiotics. An overview of the content of our review is given in Fig. 1.

## 2. Heavy metals

One of the greatest concerns about using WaW for the production of aquatic organisms for feed and food is the high potential of bioaccumulation and contamination of biomass with HMs. HMs are those chemical elements that have molecular densities higher than  $5 g cm^{-3}$ . They are highly toxic and their excessive intake could have diverse serious health effects, including carcinogenesis, decreased reproductive ability, damages to nervous, skeletal, circulatory, endocrine and immune systems of animals and humans (Abarikwu, 2013; Li et al., 2014; Morais et al., 2012). Among the various HMs, Cd, Pb, Hg and inorganic As are potentially the most toxic and are included among the ten chemicals of major public health concern (WHO, 2017). As far it is known,

**Table 1**  
Heavy metal content of some selected agro-industrial wastes and wastewaters.

	Heavy metal									References
	Cd (ppb)	Cr (ppb)	Co (ppb)	Cu (ppb)	Mn (ppb)	Pb (ppb)	Zn (ppb)	As (ppb)	Ni (ppb)	
Poultry manure/litter	0.08–38	5–2403		1.5–487	990	0.7–22	15–1063	0.5–10.4	32.6	(Markou et al., 2015; Zhang et al., 2012)
Swine manure	9			2100	2700	3.6	8800			(Kebede-Westhead et al., 2006)
Cattle manure	< 3.6	< 3.6		14–113		0.5–5.5	17–377	0.5–19		(Zhang et al., 2012)
Pig manure	0.05–203	1–43		78–1521		1.2–5.1	63–1622			(Zhang et al., 2012)
Olive oil mill wastewater		9		1	1	1	1		12	(Paredes et al., 2005)
Molasses				8.7	11		20	0.24		(Teclu et al., 2009)
Winery wastewater	50–80	200–720	110–300	200–3260	200–1740	550–1340	90–1400		200–650	(Bustamante et al., 2005)
Vinase	20–160	10–950	90–610	50–8570	730–3520	320–1740	410–2670		60–810	(Bustamante et al., 2005)
Digestates (AD)	7.1–19	120–500	16–74	650–1800	140–13,000	70–240	2800–10,000		74–210	(Valeur, 2011)

**Table 2**  
Potential range for heavy metal uptake for selected living microalgae and duckweeds.

Heavy metal	Specific HMs uptake (mg g <sup>-1</sup> dw)	Species	Reference
Cd	44.5	<i>Arthorspira platensis</i> (M)	(Murugesan et al., 2008)
	13.5	<i>Tetraselmis chii</i> (M)	(Sjahrul, 2012)
	0.02–1055	Various species (M)	(Suresh Kumar et al., 2014)
	4.7–7.7	<i>Lemna minor</i> and <i>Spirodela polyrrhiza</i> (D)	(Chaudhuri et al., 2014)
	0.28–1.56	<i>Lemna gibba</i> (D)	(Verma and Suthar, 2015)
Cr	2.5–3	<i>Lemna trisulca</i> (D)	(Huebert and Shay, 1992)
	< 0.25	<i>Arthorspira</i> (M)	(Belokobylsky et al., 2004)
	226–333	Various species (M)	(Suresh Kumar et al., 2014)
	0.6–1.2	<i>Lemna minor</i> (D)	(Wahaab et al., 1995)
	0.1–1.1	<i>Spirodela polyrrhiza</i> (D)	(Tripathi and Chandra, 1991)
Co	0.5–3	<i>Spirodela polyrrhiza</i> (D)	(Liu et al., 2017)
	0.89–1.3	<i>Chlamydomonas reinhardtii</i> (M)	(Macfie and Welbourn, 2000)
	Up to 21	<i>Lemna minor</i> (D)	(Sree et al., 2015)
Cu	6.42–7.54	<i>Chlamydomonas reinhardtii</i> (M)	(Macfie and Welbourn, 2000)
	0.5–3.25	<i>Scenedesmus obliquus</i> , <i>Chlorella pyrenoidosa</i> and <i>Closterium lunula</i> (M)	(Yan and Pan, 2002)
	1–1.8	<i>Lemna minor</i> (D)	(Wahaab et al., 1995)
Pb	Up to 5.5	<i>Lemna trisulca</i> (D)	(Prasad et al., 2001)
	4.49–5.11	<i>Pseudochlorococcum typicum</i> (M)	(Shanab et al., 2012)
	188	<i>Arthorspira platensis</i> (M)	(Arunakumara et al., 2008)
Zn	0.28–1.60	<i>Lemna gibba</i> (D)	(Verma and Suthar, 2015)
	10	<i>Lemna polyrrhiza</i> (D)	(Sharma and Gaur, 1995)
	72.1	<i>Scenedesmus subspicatus</i> (M)	(Schmitt et al., 2001)
As	0.8–4.3	<i>Lemna minor</i> (D)	(Dirilgen and Inel, 1994)
	8–20	<i>Lemna trisulca</i> (D)	(Huebert and Shay, 1992)
	0.3–1.4	<i>Dunaliella</i> sp. (M)	(Yamaoka et al., 1990)
Hg	> 1	<i>Wolffia globosa</i> (D)	(Zhang et al., 2009)
	0.5–2.2	<i>Lemna gibba</i> (D)	(Mkandawire and Dudel, 2005)
	9.2	<i>Scenedesmus subspicatus</i> (M)	(Schmitt et al., 2001)
Ni	15.1	<i>Pseudochlorococcum typicum</i> (M)	(Shanab et al., 2012)
	> 2	Not specified (D)	(Mo et al., 1989)
	0.4–0.63	<i>Chlamydomonas reinhardtii</i> (M)	(Macfie and Welbourn, 2000)
Ni	15.4	<i>Chlorella vulgaris</i> (M)	(Al-Rub et al., 2004)
	90.9	<i>Arthorspira platensis</i> (M)	(Markou et al., 2015)
	7.1–12.9	<i>Lemna minor</i> (D)	(Goswami and Majumder, 2015)
	5.5	<i>Lemna polyrrhiza</i> (D)	(Sharma and Gaur, 1995)

these HMs have no beneficial effects in humans (Abarikwu, 2013; Morais et al., 2012). In contrast, other elements regarded as heavy metals, such as Cu, Cr, Co, Mn, Ni, Se, Mo and Zn support essential physiological functions in animals and humans and are required in very small quantities (i.e. micronutrients) (Mertz, 2012). However, their intake in high quantities can likely cause health problems. Since HMs cannot be metabolized, if an organism takes them up at a rate faster than that lost by excretion, they accumulate into the animal or human body. Therefore, the maximum levels for certain HMs and other contaminants in foodstuffs and feed are regulated and legislated worldwide (see Section 6).

### 2.1. Heavy metal content in agro-industrial wastes and wastewater

Agro-industrial WaW frequently contain HMs. The species of HMs as

well their concentrations range widely depending on the WaW type, the production/processing practices and the origin of the feedstock (Markou and Georgakakis, 2011; Napan et al., 2015; Paranychianakis et al., 2015; Zhang et al., 2012). Some HMs concentrations in selected agro-industrial WaW are listed in Table 1. Most livestock manures (poultry, pig, and cattle) contain relative high amounts of HMs especially for the ones (e.g. Zn, Cu and As) that are commonly supplemented in animal feed, for growth promotion or for treatment of illnesses. HMs are usually added as highly soluble metal salts, frequently above physiological requirements and their excess amounts are excreted (Zhang et al., 2012). HMs content on manures reflects their content in feedstock, their uptake efficiency by animals, and their excretion in faeces and urine (Zhang et al., 2012).

Regarding the food processing industry, some WaW types contain relative high HMs concentrations, primarily as a consequence of the use

of industrial products (fertilizers, plant protection chemicals, processing agents etc.) that contain HMs in the agri-food production chain. The species and concentrations of HMs in AD effluents are also highly dependent on the feedstock (typically a mixture of animal manures and other organic wastes) and the specific ratio of the different WaW used. While stricter regulations are expected to minimize HMs in waste and wastewaters this is not always possible for every contaminant (Mattsson et al., 2017).

## 2.2. HMs uptake capacity

Microalgae and duckweed have been frequently considered as means for HMs removal from aqueous solutions due to their relative high affinities and sorption capacities (Chan et al., 2013; Dixit and Singh, 2014; Markou et al., 2015; Napan et al., 2015). As several reviews have adequately covered this topic (Anastopoulos and Kyzas, 2015; Kaplan, 2013; Perales-Vela et al., 2006; Rezaia et al., 2016; Suresh Kumar et al., 2015; Ziegler et al., 2016) this overview only provides a brief description. Sorption capacities of microalgae and duckweed range between 1 and 100 mg g<sup>-1</sup>; however significantly lower or higher values are also reported (Table 2). The great variation in sorption capacities is due to the different affinities of the microalgae/duckweed species for given HMs species. Sorption is governed by the electrostatic parameters of the cell walls and the organic matrices that often encapsulate algae (Rossi and de Philippis, 2016) or by the biofilms that often cover aquatic plants (Xu and Shen, 2011); the extent and composition of these, mainly polysaccharide, matrices are dependent on the organism's physiologic status (Boney, 1981; El-Sheekh et al., 2012). Therefore, it is expected that different experimental conditions that impose different stresses on the organisms will affect effective uptake and sorption of HMs. In any case, given the high affinity of microalgae and duckweed for HMs sorption and their high accumulation into the biomass, the use of agro-industrial WaW streams presents a potential for the contamination of the produced biomass with toxic HMs. However, it should be pointed out that most research on this topic has been conducted in synthetic and mono-metallic aqueous HMs solutions containing HMs concentration of one to three orders of magnitude higher than what is eventually contained in the real WaW types (Anastopoulos and Kyzas, 2015; Rezaia et al., 2016; Suresh Kumar et al., 2015), and references therein), resulting in possibly exaggerated bioaccumulation of HMs. It is expected that for HMs at the considerably lower concentrations found in real WaW, the kinetics of the HMs adsorption and uptake rates by microalgae/duckweed would be significantly distinct, with maximum accumulation values possibly lower than the ones reported for laboratory conditions. This statement is based on the HMs concentration dependent sorption isotherms where the lower the concentration of HMs in the aqueous phase the lower the equilibrium sorption. However, active uptake and intracellular bioaccumulation of HMs might not be dosage dependent as organisms can effectively remove most of the aqueous HMs from the solution (Basile et al., 2012).

The uptake capacity of microalgal/duckweed for HMs is influenced by various parameters such as, microalgae or duckweed species, HMs chemical species and concentration, co-existing ions, pH or salinity and nutrient status of the solution (Suresh Kumar et al., 2015). WaW co-existing ions or compounds (organic or inorganic), might decrease or increase HMs sorption degree through antagonistic or synergistic effects, respectively. However, the typical, and therefore more frequent observation, is that co-existing ions or other charged compounds counteract or compete with HMs decreasing the available sorption sites on the surface of biomass resulting in lower HMs uptake by the cells (Deng et al., 2006; Malik, 2004; Tsezos et al., 1996). This is of particular significance as WaW are usually rich in various ions that could interact with HMs inhibiting their uptake by cells and eventual decreasing the contamination degree of the biomass.

Moreover, especially when culturing microalgae without continuous

supplementation of CO<sub>2</sub> for pH control, the pH of the growth medium tends to increase due to photosynthesis, as OH<sup>-</sup> ions are released during uptake of CO<sub>2</sub> from bicarbonate. Under such conditions pH reaches values >>9 (Markou et al., 2014). It is expected that alkalization enhances sorption of cationic HMs (Verma and Suthar, 2015) as the functional groups on the biomass surface become deprotonated, with increased negative charges that increasingly favour the binding of metal cations (Monteiro et al., 2012). However, at high pH, the complex ionic structure of WaW creates condition favorable for ionic complexation of HMs and co-precipitation (Monteiro et al., 2012; Toumi et al., 2000), decreasing thus the availability of HMs for uptake by microalgae. More research is required to develop applied strategies for inhibiting HMs uptake by the cells. Regarding duckweed, they typically tend to equilibrate the pH at 8–8.5 (McLay, 1976; Xu et al., 2012), where HMs precipitation potential still exists, however lower than that for microalgal cultivation systems.

Studies targeting HMs sorption by microalgae and duckweed using real agro-industrial WaW are scarce (Kebede-Westhead et al., 2006; Onalo et al., 2014; Ziegler et al., 2016). More research that considers true agro-industrial WaW and realistic HMs concentrations, in the typical range of WaW, is needed in order to understand and monitor the degree of biomass contamination with HMs and to offer tools that can be deployed in the context of a food and feed safety regulatory and legislative environment.

## 2.3. Uptake mechanisms

Uptake of HMs by microalgae and duckweed follows several pathways, which could be generally categorized into: (i) cell surface sorption and (ii) intracellular accumulation (or bioaccumulation) (Fig. 2) (Basile et al., 2012; Perales-Vela et al., 2006; Suresh Kumar et al., 2015). The uptake kinetics of HMs by microalgae or duckweed could be linear or biphasic, depended on the species of HMs or the aquatic organism. In the biphasic process a first rapid removal from the solution occurs through surface sorption/precipitation followed by a second, slow phase of intracellular accumulation, i.e. the diffusion/transport of HMs into the interior of the cell (Megateli et al., 2009; Suresh Kumar et al., 2015).

### 2.3.1. Surface sorption

The term sorption refers to the adhesion of atoms, ions or molecules to a surface. Surface sorption is an electrostatic phenomenon that is governed by the totality of charged loci on the surface of cells, associated with compounds such as polysaccharides and mucilage (Boney, 1981) or cell walls components. The latter consist mainly of carbohydrates, proteins and lipids, which offer several surface functional groups such as –COOH, –OH, –PO<sub>3</sub>, –NH<sub>2</sub>, –SH that adsorb HMs through counter-ion interactions (Kaplan, 2013; Suresh Kumar et al., 2015). Surface sorption could be further divided into (i) ion-exchange, (ii) physical adsorption, (iii) complexation, (iv) precipitation, and (v) entrapment (Perales-Vela et al., 2006; Suresh Kumar et al., 2014). Surface sorption is a non-metabolic mechanism, i.e. no active metabolic process takes place for HMs uptake and therefore it is rapid, and occurs in both living and non-living cells (Kaplan, 2013). Surface sorption is a reversible process (Suresh Kumar et al., 2014) and therefore provides the opportunity of de-contamination of biomass by desorbing HMs, with salts/acids/bases or chelators.

### 2.3.2. Intracellular accumulation

Intracellular accumulation occurs also in both living and non-living cells and occurs by either passive diffusion or active transmembrane transport (Argüello et al., 2012). Active transport is a metabolically-driven process by which HMs cross the cell wall membrane through energy expending mechanisms, and thus only occurs in living cells. Intracellularly the HMs are finally bound in various organelles (in case of eukaryotic microalgae) and cell compartments (in case of



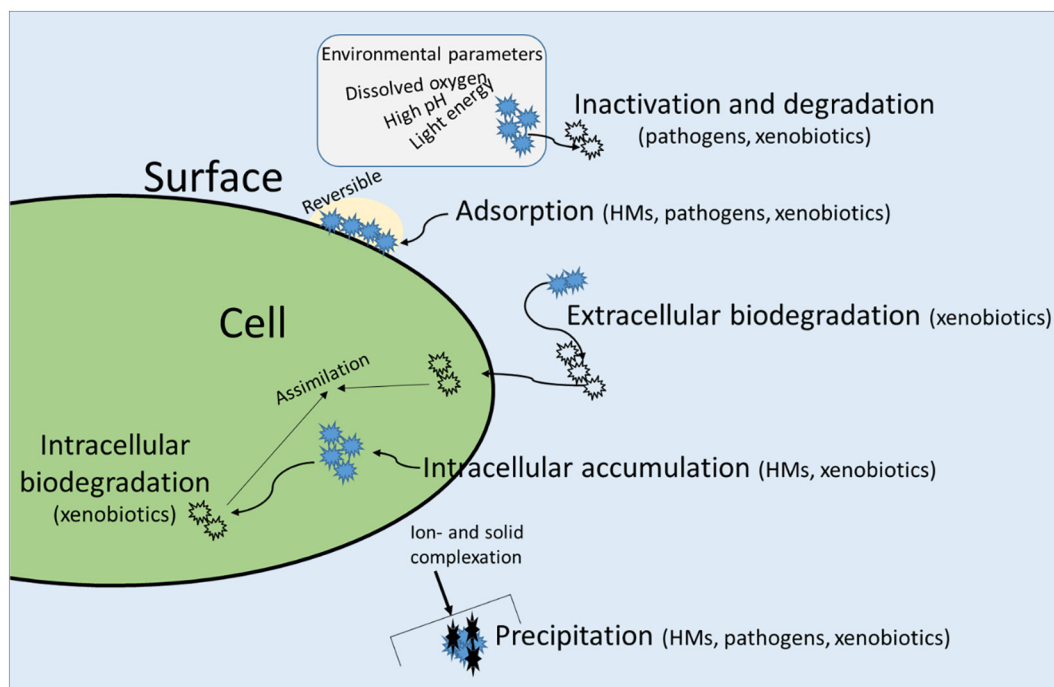


Fig. 2. Schematic representation of removal and uptake mechanisms of contaminants by microalgae or duckweed.

cyanobacteria), such as mitochondria and chloroplasts, polyphosphate bodies, vacuoles, endodermal cells, or also on surface of tissues (in case of duckweed) (Basile et al., 2012; Perales-Vela et al., 2006). Even while the cells have the ability to discriminate between non-essential and essential HMs for their metabolism (Perales-Vela et al., 2006), when the extracellular concentration of HMs is considerably higher than its intracellular concentration, then they may actively transport HMs across their cell membranes into the cytoplasm in order to store them and neutralize their potential toxicity (Monteiro et al., 2009; Perales-Vela et al., 2006). However, for both microalgae and duckweeds, if the HMs concentration exceeds a certain threshold (a function of both the HMs and the organism), it might result in inhibition of growth and lowered uptake as toxicity manifests itself in lowered metabolic activity. Intracellular accumulation is slow and irreversible, restricting the potential for de-contaminating the biomass by desorption. The overall degree of intracellular accumulation of HMs is species and strain dependent (Matsunaga et al., 1999; Suresh Kumar et al., 2015) and can reach 15–65% of the total bioaccumulated HMs. Surface adsorption or intracellular accumulation of HMs in duckweeds is insufficiently studied.

#### 2.4. Removal of heavy metals during pretreatment and biomass contamination potential

To produce biomass free of HMs it is important to identify strategies and devise methods to either reduce the concentration of HMs in the growth media, e.g. by pre-treating of WaW streams, or to manipulate the cultivation conditions in order to render HMs (bio-)unavailable and thus decreasing or nullifying contamination.

Several methods may be employed for the removal of HMs from media prior to cultivation, such as chemical precipitation, ion-exchange, adsorption, membrane filtration, coagulation and flocculation, flotation, and electrochemical treatment. However, each one of these methods has advantages and limitations (Fu and Wang, 2011). Chemical precipitation is effective only at high HMs concentrations and consumes a lot of chemicals. Ion-exchange is expensive especially when treating large volumes of wastewater containing HMs at low concentrations. Adsorption using activated carbon is also expensive, while adsorption

by low cost adsorbents, such as certain bio-residues, is a relatively new process and yet technically undeveloped. Membrane filtration technology while very efficient for HMs removal, it is, however, uneconomical for agro-industrial wastewaters that contain high organic loads and suspended solids which leads to membrane fouling and low permeate flux. Coagulation and flocculation are efficient but consume a lot of chemicals. Flotation and electrochemical treatments have high initial capital, maintenance and operation costs. In any case, the treatment method used for HMs removal prior cultivation should not remove essential nutrients (such as P and Mg), limit growth, or produce by-products that might inhibit growth or be themselves contaminants.

For AD effluents, a typical pre-treatment step that is applied in biogas plants to facilitate handling, transportation or storing of digestates, is solid/liquid separation (Möller and Müller, 2012); HMs are generally retained in the solid fraction (Tijero et al., 1990) but the degree of retention depends on the separation method and particularly on the particle size of the solids. Marcato et al. (2008) found that in anaerobically digested pig slurry most of Cu and Zn was retained in particle with sizes between 3 and 25  $\mu\text{m}$ , with only 3% bound on > 250  $\mu\text{m}$  particles. While, duckweed could be cultivated on unseparated digestates, microalgae may be cultured only in the liquid fraction of digestates, preferably filtered, as any residual solids decrease light penetration or may lead to cells clumping (Uggetti et al., 2014; Xia and Murphy, 2016). Nevertheless, solid/liquid separation may be effective for decreasing HMs in the liquid fraction of an AD effluents used for microalgae or duckweed growth. Moreover, given that usually the AD liquids are diluted at least 10 times, this may lead to a significant decrease in the concentration of HMs in the cultivation media. However, calibration work is needed to ensure that biomass is not contaminated with HMs.

Several studies demonstrated that employing chelators, such as ethylenediaminetetraacetic acid (EDTA), decreases or even eliminates the uptake of HMs by microalgae or duckweed (Huebert and Shay, 1992; Srivastava and Appenroth, 1995). Microalgae and duckweed can synthesize polypeptides with the amino acid sequence (Glu-Cys) $_n$ -Gly ( $n = 2-11$ ), known as phytochelatins (Le Faucheur et al., 2005), as a protective response to the stress induced by the presence of HMs. Chelating agents bond with metal ions, creating metal-chelate

coordination complexes, either inside or outside of the cells, which render metal ions inactive (Miazek et al., 2015). However, where HMs are adsorbed onto the suspended particles, as might be the case for WaW, the addition or synthesis of chelators could create an unbalance in the concentration of HMs between the adsorbed and soluble compartments, which can lead to desorption and possibly an increase in HMs uptake by microalgae or duckweed. Such a scenario has been confirmed for duckweed cultured in the presence of EDTA (Dipu et al., 2012).

As already mentioned in Section 2.2, a high pH could possibly decrease biomass contamination by facilitating the complexation and precipitation of HMs with other co-existing ions. However, a change of pH would also affect growth parameters and thus at this point this is only a hypothesis that requires more investigation. On the other hand, certain micronutrient HMs (e.g. Cu, Zn, Se), are essential for plant and animal metabolism. Saeid et al. (2016) demonstrated that *Spirulina* biomass enriched in Cu and Fe, through biosorption, enhanced the growth of laying hens and egg characteristics, above the results obtained by direct addition of these micronutrients to feed in a salt form. Thus, biosorption of essential HMs from WaW could be of interest for the production of feed biomass enriched in recycled micronutrients.

### 3. Pathogens

Pathogens is a broad term that covers all microorganisms that can cause disease. This includes bacteria, fungi, protozoa, worms, viruses, and infectious proteins called prions. There is an enormous heterogeneity of disease types, symptoms, transmission pathways, virulence (pathogenicity), persistence, and eco-physiology, among pathogens. Livestock manures and wastewaters commonly carry pathogens that can be transferred to biomass obtained on them. The abundance and diversity of pathogens varies significantly among WaW types and can vary among farms and geographical regions due to different production practices. Animal pathogens of concern are habitually excreted in faeces, and urine. Many pathogens are zoonotic, i.e. they may cause infections in both animals and humans, and can survive under various environmental conditions. Numerous extensive reviews have addressed livestock pathogens and their impact on animal and human health (Bicudo and Goyal, 2003; Kumar et al., 2013; Manyi-Loh et al., 2016; Mor-Mur and Yuste, 2009; Sahlström, 2003; Sobsey et al., 2006; Spencer and Guan, 2004; Turner and Burton, 1997; Ziemer et al., 2010).

#### 3.1. Pathogen content in agro-industrial wastes and wastewater

The ubiquity of pathogens in farming settings leads to the common working assumption that livestock WaW do carry pathogens (Buchanan et al., 2013; Gerardi and Zimmerman, 2004). A selected list of pathogens that can be harboured in agro-industrial WaW are presented in Table 3.

**Bacteria:** Animals and humans host an enormous population of different bacteria. Bacteria are single-cell prokaryotic microorganisms with a vast number of species, with a variety of lifestyles and morphologies (cocci/spherical, bacilli/rod-shaped, spirilla/spiral, spirochaetes/tight coils etc.). Most bacteria are not intrinsically pathogenic, however there are numerous species and strains that contain specific virulence genes. Bacteria are frequently responsible for disease outbreaks in humans and animals that originate in livestock production. The extensive use of drugs (antibiotics) in the animal production poses an additional threat to humans related to the selection and transmission of drug resistance genes and thus resistant bacteria (Bicudo and Goyal, 2003; Ray et al., 2013).

##### 3.1.1. Viruses

Particularly important are the enteric and respiratory viruses, including animal enteroviruses, rotaviruses, adenoviruses, hepatitis E

viruses, caliciviruses, reoviruses, parvoviruses and other non-enveloped viruses. They are primarily of concern to animal health because they are responsible for high morbidity and mortality and reduced production. Caliciviruses, rotaviruses, myxoviruses and hepatitis E viruses, are or may be capable of infecting humans as well (Costantini et al., 2007; Sobsey et al., 2006).

##### 3.1.2. Parasites

By definition, parasites are those organisms which live in, with or on another organism (called host) and benefits by taking up nutrients at the hosts expense. This include protozoa, helminths, and arthropods, but of significant concern are only the former two. Animal parasites that can potentially pose risks to human health include: *Acaris suum*, *Balantidium coli*, *Cryptosporidium parvum*, *Giardia lamblia*, *Microsporidia* spp., and *Toxoplasma gondii* (Sobsey et al., 2006). However, many of the parasites are of low risk for humans and others are not found in important agricultural animals (Sobsey et al., 2006).

##### 3.1.3. Prions

Prions are infectious proteinaceous agents that cause diseases of the nervous system. Prions reproduce themselves by recruiting the normal cellular isoform of the prion protein and stimulating its conversion into a disease-causing isoform (Colby and Prusiner, 2011). A significant characteristic of prions is their resistance to inactivation by ultraviolet (UV), ionizing irradiation and degradation by proteases (Aguzzi and Calella, 2009). Prion infections are typically restricted to the central nervous and lymphatic systems of infected hosts and it is probably that they are excreted with urine. However excretion of prions has been observed only in cases of chronic inflammation (Seeger et al., 2005).

##### 3.1.4. Fungi

Animal diseases caused by fungi (mycoses) are not a major concern for human health because animals are not typically a reservoir for human infections. However, some animals manure can harbour mycotic agents that could be transmitted to humans (Sobsey et al., 2006). On the other hand, agro-industrial WaW and especially of the food/feed sector, could be contaminated by fungi that produce mycotoxins (e.g. aflatoxins, zearalenone, ochratoxin) which are toxic to humans causing a wide range of effects including carcinogenicity, neurotoxicity, and developmental toxicity (Kolpin et al., 2014). Some fungi, such as *Aspergillus* sp. *Penicillium* sp., *Rhizomucor* etc. are pathogenic as well (Schnürer and Schnürer, 2006).

#### 3.2. Removal of pathogens during pretreatment

A major challenge when utilizing agro-industrial WaW for production of food and feed based on photosynthetic organisms is to reduce the pathogen content and eliminate the possibility of transmitting pathogens to humans and animals. Pathogens could be either inactivated (losing their virulence) or thoroughly removed. Due to the biological origin, there are several environmental factors that affect pathogens' survival. The most important factors include: pH, temperature, humidity, ionic and osmotic strength, competition with other flora, light (ultra-violet – UV). The mechanisms of pathogen inactivation include cell disruption, proteolysis, protein denaturation, antibiosis, antagonism and nutrient deficiencies. Many WaW treatment technologies are based on the effect of these factors on the survival of pathogens. There are three main categories of treatment methods: (i) physical, (ii) chemical, and (iii) biological. Extensive research on this matter can be found in numerous extensive reviews (Amin et al., 2013; Asghar et al., 2015; Bicudo and Goyal, 2003; Franke-Whittle and Insam, 2013; Manyi-Loh et al., 2016; Matilainen and Sillanpää, 2010; Oller et al., 2011; Särkkä et al., 2015; Sobsey et al., 2006; Verbyla and Mihelcic, 2015). We therefore offer here only a brief description of the various treatment options, with a focus, however, on AD. In practice, it is not feasible to detect every pathogen in WaW and therefore indicator

**Table 3**  
Selected pathogens found in livestock wastes and wastewater.

Pathogen	Waste and wastewater type present	Disease/symptoms	Notes
<b>Bacteria</b>			
<i>Campylobacter</i> spp.	Poultry, cattle	Gastro-enteritis, fever, headache, nausea, and vomiting	Sensitive to heat and anaerobic digestion. Not regarded as high risk. Has a low infective dose (100–800 cells can cause disease). It does not survive at a pH within the range of 1–4 or at temperatures > 47 °C. Spores remain viable in the soil for years and are claimed to be a source of infection. Very resistant.
<i>Clostridium</i> sp.	Poultry, swine, cattle	Tetanus, botulism, blackleg (clostridial myositis)/respiratory and muscular paralysis, muscle spasms	Spores remain viable in the soil for years and are claimed to be a source of infection. Very resistant.
<i>Escherichia coli</i>	Cattle, swine poultry (less)	Bloody diarrhea, vomiting, hemorrhagic colitis, haemolytic uremic syndrome	Facultative anaerobic. A strain of major concern is <i>E. coli</i> O157:H7. Grows on adverse conditions and can survive at low pH and temperatures. Can survive for long periods in soil and water. <i>E. coli</i> does not grow pH < 3.6 or in high saline conditions. Infective dose about 10 cells.
<i>Listeria monocytogenes</i>	Cattle, poultry	Listeriosis/meningitis, meningoencephalitis, brain abscess, cerebritis	Facultative anaerobic. Grows under adverse conditions and is resistant to heat and freezing.
<i>Salmonella</i> sp.	Poultry, swine, cattle	Salmonellosis/food borne enteritis, diarrhea, fever, vomiting	Facultative anaerobic. Grows at pH of 4–8, and between 8 and 45 °C. Can survive for long periods in soil and water.
<i>Yersinia enterocolitica</i>	Swine	Yersiniosis/Acute enteritis, lymphadenitis, nosodium ethema, septicemia, poliartitis and maybe death	Non-sporulated, non-capsulated; infrequent. Grows at pH 4–10 and at 4–43 °C.
<b>Viruses</b>			
Porcine circovirus	Swine	Porcine dermatitis and nephropathy syndrome, porcine respiratory disease complex, postweaning multisystemic wasting syndrome	It is heat (70 °C) and chemical resistant. Can survive for long periods. Anaerobic digestion reduces infectivity.
Coronavirus	Many animals		Sensitive to stresses. Does not survive for long periods.
Rotavirus	Many animals	Acute viral gastroenteritis/diarrhea	Potential zoonotic. Resistant to detergent and many antiseptics. Anaerobic digestion and UV reduces infectivity.
Hepatitis E virus	Swine, sheep, poultry	Liver disease/anorexia, nausea and vomiting, hepatomegaly	Zoonotic. Persistence characteristics are not known.
Influenza	Many animals	Flu	Zoonotic. Sensitive to heat, irradiation, detergents and oxidizing agents.
<b>Parasites</b>			
<i>Ascaris suum</i>	Many animals	Ascariasis	Parasitic nematode, zoonotic; eggs survive under anaerobic stabilization (> 80% viability after 20 days).
<i>Giardia</i> sp.	Many animals	Giardiasis/diarrhea, cramps, flatulence.	Flagellate protozoan parasite, zoonotic. Very low infection dose. Cysts survive for long periods. In water oocysts survive < 14 days at 25 °C.
<i>Cryptosporidium parvum</i>	Many animals	Cryptosporidiosis/diarrhea, dehydration, nausea, vomiting	Very low infection dose (132 oocysts) Oocysts are resistant to disinfectants Relative sensitive to heat.
<b>Prions</b>		Nervous system disease such as Creutzfeldt–Jakob disease	Resistant to high temperature, in general difficult to be disinfected.

Data summarized from (Bicudo and Goyal, 2003; Costantini et al., 2007; Kumar et al., 2013; Manyi-Loh et al., 2016; Sahlström, 2003; Spencer and Guan, 2004; Ziemer et al., 2010).

pathogens are frequently used as risk indicators for putative presence of pathogens.

### 3.2.1. Physical methods

This category includes pasteurization, UV irradiation and filtration. Pasteurization entails increasing the temperature, typically to 70 °C, for several minutes (30–60 min). It is effective and eliminates most pathogens (e.g. bacterial indicators, *Ascaris suum* eggs, swine vesicular disease virus), however it does not eliminate spore-forming bacteria, like *Clostridium* spores or viruses such as porcine parvovirus (Bagge et al., 2010; Sahlström et al., 2008). Increasing the pasteurization temperature at 90 °C could result in a more reliable inactivation of bacteria, heat-resistant viruses and parasites (Martens and Böhm, 2009). It is however an energy intensive process that could be economically feasible when waste thermal energy is available, such as in biogas production plants. UV irradiation can achieve high reduction of enteric bacterial and protozoan pathogens (2–5 log<sub>10</sub>), however some viruses are relatively resistant to UV irradiation (Bilotta and Kunz, 2013; Sobsey et al., 2006). UV irradiation does not generate any unwanted residuals and does not alter the nutritional composition of WaW. Although filtration (ultra- and nano-filtration) is effective to remove pathogens (even prions) (Yunoki et al., 2008), its application for large volumes of WaW, at an industrial scale as required for effective

biomass production, is hindered by the presence of the various suspended and dissolved solids.

### 3.2.2. Chemical methods

This category includes alkaline treatment, chlorination, ozonation, and advanced oxidation processes. Alkaline treatment involves the addition of alkali, like KOH, CaO or Ca(OH)<sub>2</sub>, and mixes with other materials like ash, to reach a pH > 12. The most frequent alkaline treatment is lime stabilization (CaO or Ca(OH)<sub>2</sub>). This is effective for most pathogens, with reduction of fecal coliforms, *Salmonella* sp., helminth eggs and protozoan oocysts up to 98–99.99% that can be achieved after a few hours of stabilization (Sobsey et al., 2006; Viancelli et al., 2015; Wong and Selvam, 2009). However, alkaline stabilization can also precipitate nutrients such as phosphorus. Combining alkaline treatment with heat favours alkaline hydrolysis, a confirmed reliable method for inactivation of pathogens (Kaye et al., 1998). Hydrolysates thus obtained might contain amino acids and sugars that may be directly assimilated by microalgae or duckweeds (Markou et al., 2014) offering an attractive source of organics for mixotrophic or heterotrophic growth. Chlorination is the most cost-effective process for wastewater disinfection (Amin et al., 2013; Rodríguez-Chueca et al., 2015); however the main drawback is the formation of toxic and carcinogenic by-products. Moreover, although chlorination is very



effective for inactivation of bacteria it is less effective for reduction of viruses and protozoa (Sobsey et al., 2006). Ozonation is effective for inactivation of bacteria, viruses and protozoa; however organic matter in WaW can inactivate ozone which limits the efficacy of the method leading to requirements for high ozone doses ( $1\text{--}2\text{ g L}^{-1}$ ) (Sobsey et al., 2006; Watkins et al., 1997; Wu et al., 1998). Ozonation does not alter the nitrogen and phosphorus content of the wastewater (Watkins et al., 1997; Wu et al., 1998). The use of advanced oxidation processes for agro-industrial WaW could be more appropriate since organic matter is degraded during the process and does not interfere with the disinfection ability. Advanced oxidation processes are effective for disinfection giving bacterial reduction higher than  $3\text{ log}_{10}$  (Rodríguez-Chueca et al., 2015) and have been widely studied for agro-industrial WaW (Deng and Zhao, 2015; Matilainen and Sillanpää, 2010; Oller et al., 2011; Wagner et al., 2002). However, more research is needed around the formation of potentially toxic intermediate products (Oller et al., 2011; Särkkä et al., 2015).

### 3.2.3. Biological methods

Agro-industrial WaW are treated mainly by biological methods, such as stabilization lagoons and AD. Other biological methods such as aerobic digestion, biofilters, activated sludge or constructed wetlands are not commonly employed for agro-industrial WaW. Stabilization lagoons (aerated, anaerobic, facultative or multiple configurations) offer low-cost options for WaW treatment. Especially multiple stabilization ponds are more effective in pathogens reduction ( $2\text{--}6\text{ log}_{10}$ ) than single ponds ( $1\text{--}3\text{ log}_{10}$ ) (Sobsey et al., 2006; Viancelli et al., 2013). However, they require long retention times ( $> 3$  months), their performance is not consistent, and depend significantly on environmental parameters (Sobsey et al., 2006). Moreover, due to the long retention times required high loss of nutrients could occur; over 80% of N could be lost through ammonia volatilization (Rockne and Brezonik, 2006). Among the biological methods for agro-industrial WaW treatment, AD is increasingly gaining interest because of the simultaneous treatment of the WaW and the production of biogas, which is an energy carrier that can be used to produce electricity/thermal energy or transportation fuels (biomethane) (Weiland, 2010). AD may be performed either in the psychrophilic (temperature of the environment), mesophilic ( $30\text{--}40\text{ }^{\circ}\text{C}$ ) or thermophilic ( $45\text{--}65\text{ }^{\circ}\text{C}$ ) temperature ranges. Most frequently AD is carried out in the mesophilic range, as it is more robust compared to the thermophilic range digestion. The latter exhibits uncertainty in methanogenesis, especially when the ammoniacal nitrogen concentration of the waste liquor is relative high (Moset et al., 2015; Yenigün and Demirel, 2013). Thermophilic AD is nonetheless more effective for pathogen reduction (Table 4). Nevertheless, thermophilic digestion does not inactivate spore-forming pathogens of *Clostridium* or *Bacillus* (Bagge et al., 2010; Sahlström, 2003). An increase in organic acids (volatile fatty acids, such as acetate) in the digestion liquor may substantially reduce spore-forming pathogens (Salsali et al., 2008; Xu et al., 2015) and thus a two-phase thermophilic/mesophilic configuration could be applied to address such pathogens (Huyard et al., 2000). Such an approach might also overcome the instabilities of the thermophilic reactors. In practice, a pasteurization stage ( $70\text{ }^{\circ}\text{C}$  for 1 h) either before or after the mesophilic AD is included to ensure the hygiene of the effluents (Sahlström, 2003). Still, pasteurization does not fully inactivates all spore-forming microorganisms (bacteria or fungi) (Bagge et al., 2010; Sahlström, 2003; Schnürer and Schnürer, 2006) and it was proposed to combine it with alkaline hydrolysis for increasing the reliability of disinfection (Kaye et al., 1998). In general, the wide diversity of pathogens renders WaW difficult to disinfection via a single technology, and often a combination of technologies is required (Viancelli et al., 2013). Wastewater treatment research shows that pathogens partition between the solid and the liquid phase at variable proportions is a function of the treatment option and the status of the abiotic parameters in the treatment system (van der Drift et al., 1977; Zhang and Farahbakhsh, 2007). Nevertheless, studies directly focusing

on the distribution of pathogens between the solid and liquid fractions after separation are scarce. It was found however, that most indicator pathogens were retained in the liquid fraction (Liu et al., 2017). Additionally, improved animal management and housing techniques could also contribute in reducing pathogen load in animal WaW. Such techniques include vaccination and antibiotic therapy, adjustments of animal diets, on-farm hygienic and sanitation actions, and livestock housing management (Sobsey et al., 2006).

### 3.3. Contamination potential of produced biomass with pathogens mechanisms of contamination

A question of particular interest regarding the growth of aquatic organisms in agro-industrial WaW is whether the various pathogens harboured by WaW may contaminate biomass and what are the mechanisms of contamination. There are numerous studies investigating the interaction of microalgae or duckweeds with bacteria (Fuentes et al., 2016; Underwood and Baker, 1991), viruses (Short, 2012) or protozoan grazers (Tillmann, 2004) and their symbiotic, antagonistic or parasitic relationships. However, there is insufficient direct evidence on whether microalgae or duckweeds might host human or animal pathogens. Considering the known capacity of viruses or bacteria to interact with particles in suspension, it can be hypothesized that potential mechanisms for contamination of biomass with pathogens would involve adsorption of pathogens on microalgal/duckweed biomass surface (Fig. 2) through the variably distributed hydrophilic and hydrophobic loci on both the surface of bacteria and the algae or duckweeds (Marshall, 1985; Verbyla and Mihelcic, 2015) and the role of the ionic composition, i.e. pH and ionic strength, in modifying the net expression of these charges. The geometry of the interacting surfaces is also critical, and as this varies among microorganisms it affects the effective range of the electrostatic interactions, and the thus strength of adsorption. Production of charged compounds, such as polysaccharide capsules, or certain proteins (e.g. adhesins) will enhance the likelihood and strength of attachment. Often such compounds are produced under non-ideal conditions and thus management of the growth conditions might modify the attachment kinetics.

Bacterial pathogens (such as *Salmonella* and *E. coli*) can also invade plants via roots or shoots (Cooley et al., 2003; Zhang et al., 2010) and establish themselves within intercellular spaces, in plant tissues (Ávila-Quezada et al., 2010). As in the case of HMs, it can be speculated that the inter/intracellular contamination with pathogens would be more difficult to addressed, whereas surface bound pathogens could be desorbed or treated for their inactivation.

On the other hand, it has been frequently reported that during microalgae or duckweeds cultivation on wastewater certain indicator bacteria were significantly reduced (Heubeck et al., 2007; Iqbal, 1999; Posadas et al., 2015; Schumacher et al., 2003). For example, *Staphylococcus aureus*, *Escherichia coli*, and *Enterococcus faecalis* were reduced by  $3\text{--}4\text{ log}$  in cultures of the microalga *Scenedesmus* sp. (Al-Gheethi et al., 2017). It is unclear, however, if such reduction was attained through pathogen death, inactivation, or is an artefact of the testing procedure. In general, potential mechanisms for the removal/inactivation of pathogen in microalgal cultures systems include (i) inactivation through solar irradiation (UV), (ii) drastic shift in pH, either due to the photosynthesis linked pH increase ( $> 9\text{--}9.5$ ), or due to rapid acidification to pH of  $1\text{--}2$ , as obtained when extremophiles such as *Galdieria* sp. are cultured in wastewaters (Henkanatte-Gedera et al., 2017), (iii) photosynthesis driven increases in dissolved oxygen concentrations in the cultivation media, and (iv) the production and excretion of antibacterial substances by algae (Heubeck et al., 2007; Posadas et al., 2015). In case of duckweeds, many of these pathogen removals methods are expected to be unpractical or less effective as the floating duckweed plants shade the cultivation media minimizing solar irradiation and, given that photosynthesis occurs above the water-air interface, also minimizes any drastic effect on the pH or dissolved oxygen

**Table 4**  
Inactivation of selected pathogens during anaerobic digestion.

Pathogens	Disinfection method	Reduction (log <sub>10</sub> )	Time of complete inactivation	References
<i>Campylobacter</i> spp	Thermophilic anaerobic digestion		> 24 h@53 °C	(Wagner et al., 2008)
<i>Listeria monocytogenes</i>	Thermophilic anaerobic digestion		> 24 h@53 °C	(Wagner et al., 2008)
<i>Listeria monocytogenes</i>	Mesophilic anaerobic digestion	2.23@35 °C > 14 d		(Horan et al., 2004)
<i>Clostridium</i> sp.	Thermophilic anaerobic digestion		Inactivation rate 0.188–2.681 CFU/d @ 55 °C	(Xu et al., 2015)
<i>Escherichia coli</i>	Mesophilic anaerobic digestion	1.66@35 °C > 14 d		(Horan et al., 2004)
<i>Escherichia coli</i>	Thermophilic anaerobic digestion	Decimal reduction 10 min at 55 °C		(Aitken et al., 2007)
<i>Escherichia coli</i>	Thermophilic anaerobic digestion		< 4 d@52.5 °C	(Pandey and Soupir, 2011)
Fecal enterococci	Anaerobic digestion	4 log <sub>10</sub> after 300 h@35 °C or 1–2 h@ 55 °C		(Shilton et al., 2008)
<i>Salmonella</i> sp.	Mesophilic anaerobic digestion	2.23@35 °C > 14 d		(Horan et al., 2004)
<i>Ascaris suum</i>	Thermophilic anaerobic digestion		> 6 h@49 °C < 0.5 h @ 55 °C	(Aitken et al., 2005)
<i>Giardia muris</i>	Anaerobic digestion	3 log <sub>10</sub> after 20.5 h@37 °C or 11 min@ 55 °C		(Van Praagh et al., 1993)
<i>Cryptosporidium parvum</i>		2 log <sub>10</sub> after 10 d@37 °C or 2 d@55 °C		(Kato et al., 2003)
Porcine parvovirus	Anaerobic digestion	MGRT 11–12 h@55 °C		(Lund et al., 1996)
Bovine enterovirus	Anaerobic digestion	MGRT 23 h@35 °C, or < 0.5 h@55 °C		(Lund et al., 1996)

MGRT: minimum guaranteed retention time.

1 log<sub>10</sub> corresponds to 90% inactivation, 2 log<sub>10</sub> to 99%, 3 log<sub>10</sub> to 99.99% etc.

of the media (pH 8–8.5) (Dewedar and Bahgat, 1995; McLay, 1976; Smith and Moelyowati, 2001; Xu et al., 2012). There are some indications however, that duckweeds cultivated in municipal (Moyo et al., 2003) or even hospital-based wastewater (Islam et al., 2004) might be safe to use as feed because it was found that they were not contaminated with pathogens. More insight into such observations is needed to support any decision making.

#### 4. Xenobiotics

Besides HMs and pathogens, agro-industrial WaW contain also several other hazardous compounds that could contaminate the produced biomass of microalgae/duckweed. The most important of these, that will be briefly discussed herein, are pharmaceuticals (steroidal hormones, antibiotics and parasiticides), mycotoxins and dioxins.

##### 4.1. Pharmaceuticals

###### 4.1.1. Steroidal hormones

Hormonal growth promoters are often added to livestock feed to increase feed efficiency and accelerate weight gain. These are mainly estrogens, testosterone and progesterone, and various synthetic hormones that regulate growth and development of animals (Ray et al., 2013). Once excreted by livestock into the environment these compounds display endocrine disrupting effects (Combalbert and Hernandez-Raquet, 2010; Ray et al., 2013). Among the estrogens, 17 $\alpha$ -estradiol (E2 $\alpha$ ), 17 $\beta$ -estradiol (E2 $\beta$ ), and estrone (E1) account for > 90% of the excreted estrogens by cattle, while E2 $\beta$  is the most prevalent in poultry (Table 5) (Ray et al., 2013). In general, estrogens concentrations vary significantly (Table 5) with age, diet, and health status of the animals, as well by the manure/urine collection and handling practices (Combalbert and Hernandez-Raquet, 2010; Ray et al., 2013).

###### 4.1.2. Antibiotics

Besides their therapeutic use antibiotics are also used as growth promoters (Hughes and Heritage, 2004; Van Boeckel et al., 2017). Most antibiotics though are poorly absorbed by the gastrointestinal tract and therefore are largely (17–90%) excreted in faeces and urine (Van Boeckel et al., 2017) either unmodified or as active metabolites

**Table 5**

Estrogen excretions by selected animals and estrogen content in their manures. Adapted from (Combalbert and Hernandez-Raquet, 2010; Ray et al., 2013).

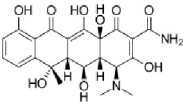
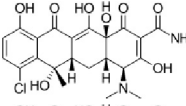
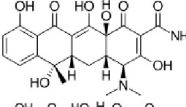
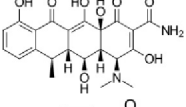
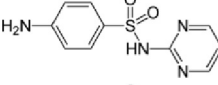
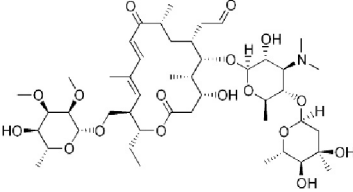
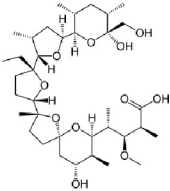
Species	Fecal excretion (μg d <sup>-1</sup> per animal)	Urine excretion (μg d <sup>-1</sup> per animal)	Droppings (μg d <sup>-1</sup> per animal)	Daily excretion (μg d <sup>-1</sup> per animal)	Estrogen content in manure (μg kg <sup>-1</sup> )
Cattle	> 30–360	15–180		45–540	E2 $\alpha$ ; < 1.1–1113 E2 $\beta$ ; < 1.9–485 E1; < 5–865
Swine	14–270	100–2000		120–2300	E2 $\alpha$ ; 9 E2 $\beta$ ; 115 E1; 243
Poultry			2.5–6		E2 $\alpha$ ; 93 E2 $\beta$ ; 150 E1; 44

(epimers or isomers) of the parent compounds (Massé et al., 2014; Sarmah et al., 2006). In some cases, such in *N*-acetyl-sulfonamides, the metabolized fraction contained in manures can be transformed again into parent compound (Mohring et al., 2009). The most serious threat associated with the use and environmental contamination with antibiotics is the rise of new strains of antibiotic resistant bacteria (Sarmah et al., 2006). The content of antibiotics in urine, that enters manure, (fresh or stored) varies widely (Table 6) but typically ranges between 1 and 10 mg L<sup>-1</sup> or mg kg<sup>-1</sup> (Massé et al., 2014).

###### 4.1.3. Parasiticides

Parasiticides refer to the application of chemicals to treat and control *endo*- or *ecto*-parasites (organisms that live on the inside or outside of its host, respectively), such as flies, lice, acari, mosquitoes, worms, protozoa and coccidia. There is a wide range of chemical compounds employed as antiparasiticides, such as chlorinated hydrocarbons, organophosphates and carbamates, synthetic pyrethroids, amides, macrocyclic lactones and benzylphenyl ureas (Bártíková et al., 2016; Khan et al., 2008). Some parasiticides, such as synthetic pyrethroids are of concern for human health as they are known disruptors of the endocrine system (Coleman et al., 2013; Khan et al., 2008). Given their diversity in chemistry they vary considerably in their excretion rates by

**Table 6**  
Antibiotics content in fresh or stored animal manure/urine and their removal during anaerobic digestion (adapted from (Massé et al., 2014)).

Antibiotic	Chemical structure	mg L <sup>-1</sup> or mg kg <sup>-1</sup>	Removal (%)	References
Oxytetracycline		0.4–354	59–85%	(Álvarez et al., 2010; Massé et al., 2014)
Chlortetracycline		1–139	27–90	(Álvarez et al., 2010; Gartiser et al., 2007; Massé et al., 2014)
Tetracycline		30–98	–	(Massé et al., 2014)
Doxycycline		37	–	(Massé et al., 2014)
Sulfadiazine		7.1	70–100	(Massé et al., 2014; Mohring et al., 2009)
Tylosin		0.11–8.1	100	(Massé et al., 2014; Mitchell et al., 2013)
Monensin		120	60	(Gartiser et al., 2007; Massé et al., 2014)

animals. Some organophosphate and carbamate compounds might be efficiently metabolized, while other compounds, such as synthetic pyrethroids, benzylphenyl ureas, macrocyclic lactones and fluzuron, are excreted without much change in mass (Khan et al., 2008). Their discontinuous use in livestock, seasonal or as needed, leads to large variations in their concentrations in manures. For example, Coleman et al. (2013) found that the concentration of abamectin, ivermectin and doramectin in fresh or stored manure range between < 1 and 36 µg kg<sup>-1</sup>.

#### 4.2. Mycotoxins and dioxins

Mycotoxins or dioxins are also potential contaminants but there is little known about their presence in manure/urine. Mycotoxins could be a concern if contaminated agro-food wastes and by-products are used as nutrient source for growing microalgae/duckweed. Fungi can easily contaminate agro-food products generating mycotoxins, such as ochratoxin, zearalone, deoxynivalenol, which can have a wide range of effects including carcinogenicity, neurotoxicity, and developmental toxicity (Ajila et al., 2012; Kolpin et al., 2014). Dioxins are halogenated organic compounds generated in industrial processes. Many dioxins are persistent and have become abundant in the environment; livestock is exposed to dioxins mainly by consuming feed contaminated by airborne dioxins (Khan et al., 2008). It is unclear if these are of great significance in liquid growth systems, but they can easily contaminate biomass post-growth.

#### 4.3. Cyanotoxins and microcystins

An additional category of xenobiotics that possess a health risk, not carried in WaW, is the presence of cyanotoxins and microcystins produced by cyanobacterial species that invade and contaminate microalgal and duckweed cultures. Open pond production systems are susceptible to such contaminations. Cyanotoxins and microcystins are highly soluble and have numerous toxic effects causing gastroenteritis, allergic and inflammatory reactions, liver injury and even death (Bláha et al., 2009; Dawson, 1998). There have been reports about contaminated commercial algae dietary supplements with various toxins at levels above the tolerable daily intake values (Roy-Lachapelle et al., 2017).

#### 4.4. Removal of chemical compounds during pretreatment

Many technologies employed for pathogen removal could be also used for the removal of contaminants. Briefly, some physico-chemical technologies that have been investigated for the removal of pharmaceuticals and other chemical compounds are: adsorption, chemical advanced oxidation processes (ozonation, Fenton oxidation, UV treatment and ionizing irradiation) (Wang and Wang, 2016). The removal efficiencies range significantly depending on the chemical compound, the type of the WaW and the operation parameters (Wang and Wang, 2016). However, each of these technologies has various limitations (see Section 2.4) and are not easily implementable in practice. Efficiency of AD for removal of chemical compounds has been shown to vary widely, from negligible to almost complete removal, and is mainly a function of

the chemical characteristics of the compound in question (Stasinakis, 2012). Nevertheless, few studies focused on the value of AD for the removal of chemical compounds from agro-industrial wastes (des Mes et al., 2008; Zhao, 2008).

#### 4.4.1. Steroid estrogens

Under anaerobic conditions (anaerobic digesters, anaerobic storage or anoxic lagoons) E1 is reduced to E2 ( $\alpha$  and  $\beta$ ) and further to E3. The total estrogen content is generally removed only to a small degree (< 22%) (Combalbert et al., 2012; des Mes et al., 2008; Zhang et al., 2014b; Zheng et al., 2012), although, removals higher than 50% have also been reported (Paterakis et al., 2012). Hormones have low Henry's constants (in the order of magnitude of  $10^{-11}$  (atm L<sup>-1</sup> mol<sup>-1</sup>)) and have hydrophobic properties (octanol/water partition coefficient,  $K_{ow}$ , of about 2.41–4.01) and consequently they display a great affinity for adsorption onto (bio)solids (Chawla et al., 2014). It is expected therefore that after solid/liquid separation of digestates estrogens will be largely retained within the solid fraction, thus reducing the amount in the liquid phase that would be used for cultivation of algae and duckweeds. Moreover, it might be possible that they form sulfate-conjugated forms in manures, that are more recalcitrant to biodegradation than the free estrogen forms (Combalbert et al., 2012; des Mes et al., 2008). It seems that aerobic conditions, either as aerated reactors or by aerobic composting, are necessary for a significant (> 70%) removal of estrogens (Zhang et al., 2014b; Zhao, 2008).

#### 4.4.2. Antibiotics

The fate of veterinary antibiotics during AD of manures have been studied by various researchers (Álvarez et al., 2010; Arikan, 2008; Arikan et al., 2006; Gartiser et al., 2007; Mitchell et al., 2013; Mohring et al., 2009). Table 6 lists the impact of AD on the removal rates of antibiotics. The degree of degradation varies significantly and depends on the antibiotic compound and the AD parameters. A general trend observed in studies dealing with oxytetracycline, chlortetracycline, sulfonamides and tylosin is that they are relatively quickly transformed into intermediate products, which are then either further degraded or persist. Persistence of antibiotics is probably related to their capacity to be adsorbed onto (bio)solids increasing their stability (Álvarez et al., 2010); e.g. the water soluble fraction of oxytetracycline was degraded significantly more (up to 85%) than the solid bound fraction (Álvarez et al., 2010). Tylosin was completely removed after 4 days; however the two degradation products formed persisted at least 40 days with 20–50% adsorbed onto solids and the rest remaining in the liquid phase (Mitchell et al., 2013). In some cases, like for chlortetracycline, the concentrations of water-soluble degradation products increased about 2-fold during digestion (Arikan, 2008). Any concern regarding these degradation by-products is directly related to whether the transformation products display the same or different antibiotic activities.

#### 4.4.3. Parasiticides, mycotoxins and dioxins

Investigations into the degradation of parasiticides, mycotoxins and dioxins during AD of agro-industrial WaW are scarce. Kupper et al. (2008) found that about 50% of parasiticides originating in feedstock were 96% to 100% degraded, about 35% were degraded at a proportion of 51–95%, with the rest degraded at proportions of < 50%. After the solid/liquid separation of the digestates it was found that parasiticides end up preferentially in the liquid fraction. In the study of Salati et al. (2014) 69% to 87% of the mycotoxin aflatoxin B1 was degraded under batch mode of AD while at semi-continuous mode the degradation was 42% in average. Dioxins are considered to be highly resistant to biodegradation probably due to their low water solubility and high  $K_{ow}$  coefficients (Kulkarni et al., 2008). After 6 months of AD the concentration of some dioxins (polychlorinated dibenzo-p-dioxins and dibenzofurans) has been shown to remain unchanged while some other dioxins (3-chlorophenol and 3,4-dichlorophenol) were newly generated; however, pentachlorophenol concentration decreased 57%

(Najmanová et al., 2014).

### 4.5. Uptake mechanisms and biomass contamination potentials

During cultivation of microalgae/duckweed in media that contain xenobiotics, various removal mechanisms take place such as adsorption onto (bio)solids, cell uptake, volatilization, photodegradation and biological degradation and transformation (Wang et al., 2017; Zhang et al., 2014a). However, the type of removal mechanism depends on the physico-chemical characteristics of the compounds resulting in a significant variation in the degree and type of removal or uptake (Fig. 2) (Matamoros et al., 2015; Wang et al., 2017; Zhang et al., 2014a).

#### 4.5.1. Steroid estrogens

During microalgae/duckweed cultivation estrogens are converted from one form into another; E1, E2 and EE2 can be interconverted rapidly, during a few hours of cultivation, while estrone (E1) is transformed into estriol and after an extended period (> 7 days) is further degraded into unknown lipophilic products (Lai et al., 2002; Shi et al., 2010; Zhang et al., 2014c). Among the removal mechanisms, it was shown that photodegradation and direct oxidation accounted for a small fraction of the total estrogen removal (Maes et al., 2014; Shi et al., 2010; Zhang et al., 2014c). On the other hand, even as the final removal of total estrogen can reach over than 85%, it was shown that the estrogenic activity of the medium was not reduced suggesting that the degradation products displayed the same or higher estrogenic activity compared to the parent compounds (Zhang et al., 2014c). Estrogen sorption onto biomass can range between 4 and 58% and might depend on the species of the organism, the estrogen concentration, or other cultivation parameters (Hom-Diaz et al., 2015; Zhang et al., 2014c). The degree of intracellular concentration of estrogen has not yet been extensively investigated; however, it was shown that the microalga *Desmodesmus subspicatus* incorporated around 23% EE2 from its surrounding medium after 24 h, with no further uptake. After re-incubation of contaminated cells in clean medium a portion of EE2 was desorbed, but 30% of the initially incorporated estrogen remained in the microalgal biomass (Maes et al., 2014), suggesting, at least partially, a gradient driven adsorption onto cell surfaces.

#### 4.5.2. Antibiotics

Investigations into growing microalgae/duckweed in the presence of veterinary antibiotics are very scarce. When studied, microalgae and duckweed have been shown to be successfully employed for the removal of various antibiotics (Wang et al., 2017; Zhang et al., 2014a). The microalgal uptake, along with photodegradation (contributing around 21%) eliminated > 88% of the compound (Santaeufemia et al., 2016). However, living cells of the microalga *Phaeodactylum tricorutum* have been shown to have a sorption capacity for oxytetracycline of about 29 mg g<sup>-1</sup> (Santaeufemia et al., 2016).

#### 4.5.3. Parasiticides, mycotoxins and dioxins

These categories include numerous compounds with diverse physico-chemical characteristics and persistence. There is a vast literature about their toxic effects on phytoplankton and plants, however fewer reports exist on their removal using microalgae or duckweed, and research about the contamination of microalgal or duckweed biomass is scarce (Dosnon-Olette et al., 2010; Olette et al., 2008; Weiner et al., 2004). Microalgae and duckweed might degrade some parasiticides like deltamethrin (Muir et al., 1985) or diazinon (Kurade et al., 2016) forming several other degradation by-products. There are no available data regarding mycotoxins, only one report that indicates that algae (unspecified) were not able to degrade aflatoxin (Ciegler et al., 1966); there are only a few reports on the ability of microalgae to degrade dioxins and various other persistent organic pollutants (Cepoi et al., 2016). Concerning the biomass contamination, it was shown that the pyrethroid deltamethrin parasiticide was rapidly accumulated by



**Table 7**

Maximum levels of the most significant contaminants in feed and food set by *Codex Alimentarius*, FAO (CAC, 1995).

Ochratoxin A ( $\mu\text{g kg}^{-1}$ )	5
As ( $\text{mg kg}^{-1}$ )	0.01–0.5
Cd ( $\text{mg kg}^{-1}$ )	0.05–2
Pb ( $\text{mg kg}^{-1}$ )	0.01–1
Hg (methylmercury) ( $\text{mg kg}^{-1}$ )	0.5–1
Sn	50–250

*Lemma* sp. during the initial 24 h reaching 253 and 308  $\text{ng g}^{-1}$  dry weight, and that this dropped to < 50% after 3 days due to degradation (Muir et al., 1985). Weiner et al. (2004) found that a correlation between atrazine uptake and microalgal species-sensitivity to the compound exists. The more sensitive species accumulated more atrazine than less sensitive species. It was hypothesized that less sensitive species may actively pump atrazine out of the cell or may degrade it intracellularly.

## 5. Contamination potentials of extracted products

As was mentioned before, a variety of high-value metabolites e.g. pigments, fatty acids, proteins, antioxidants etc. could be extracted from microalgae and duckweed. Ruiz et al. (2016) conducted a techno-economical evaluation of microalgae feasibility and concluded that nowadays the production of high-value metabolites could be profitable. Thus, it is of interest to identify if the production of high-value metabolites could be coupled with the use of WaW in the context of circular economy, while on the other hand might reduce biomass production costs. However, the potential of transferring contaminants, e.g. HMs, pathogens or xenobiotics that originate in WaW, to the extracted products is possible and thus a concern. There is however a paucity of studies demonstrating this potential, indicating that there are research opportunities on this topic.

A first key step for the extraction of the metabolites is the disruption of cells to facilitate access on the intracellular ingredients. Microalgae and duckweed have relatively rigid cell walls (compared to bacteria or yeast) requiring harsher techniques for the disruption of cell walls or tissues. Cell disruption methods may be grouped into two categories: (1) mechanical and (2) non-mechanical (Günerken et al., 2015): *mechanical* methods include (i) bead milling, (ii) high-pressure homogenization, (iii) high-speed homogenization, (iv) ultrasonication, (v) microwave, and (vi) pulsed electric field treatments; *non-mechanical* methods include (i) enzymatic cell lysis and (ii) chemical cell disruption (Günerken et al., 2015). Some cell disruption technologies (like the chemical ones) can also simultaneously extract some target metabolites. The core stage of metabolite extraction is conducted mainly by chemical methods (organic solvents, alkali or acid salts, polymer-salts, super- or subcritical fluids extraction etc.) that might be assisted by mechanical methods (such as microwave) followed by product purification using chromatographic technologies (ion-exchange, gel filtration, expanded bed absorption etc.), membrane separation (micro-, ultra-, and nano-filtration or reverse osmosis) or chemical (caustic refining etc.) (Cuellar-Bermudez et al., 2015; Günerken et al., 2015; Gerardo et al., 2014). The method applied for cell disruption, metabolite extraction and purification depends on the physico-chemical characteristics of tissues and the cell membranes and the target compound(s), the latter determined mainly by their hydrophilic (phycobilins, some proteins, sugars etc.) or hydrophobic (lipid-based pigments, fatty acids etc.) properties. Some cell disruption methods such as high-speed homogenization (Dong et al., 2015) or bead milling (Doucha and Lívanský, 2008) could further contribute to cell lysis, however it is expected to have little to no effect on HMs or xenobiotics. Given that contaminants have also hydrophilic or hydrophobic properties they could be extracted along with the target compound(s). It seems that the

purification step might be the most critical because here highly efficient separation technologies could be applied in order to obtain a pure and safe product for consumption. More research to elucidate these points is critical.

## 6. Regulations regarding feed and food safety and the treatment of agro-industrial wastes and wastewater

Food and feed can become contaminated by various causes and processes during their production and this may pose a risk to human or animal health. Therefore, most jurisdictions regulate the presence of contaminants or unwanted compounds in feed and food, setting various recommendations, regulations, and standards to ensure their quality and safety. While there are major commonalities, the particularities of the local regulatory systems offer a wide range of variability in the regulations and their implementation strategies. The Food and Agriculture Organization (FAO) of the United Nations has set a series of standards and recommendations in the *Codex Alimentarius* (Codex Stan 193-1995) to address the presence of contaminants, such as HMs and toxins in food and feed and listing their maximum levels (ML) (Table 7 lists the ML of most significant contaminants). FAO *Codex Alimentarius* points out the significance of the Good Agricultural Practice (GAP) and Good Manufacturing Practice (GMP) in order to achieve a low as reasonably presence of contaminants in feed and food. GAP and GMP includes the following activities to prevent or to reduce contamination of feed and food (CAC, 1995): “(i) preventing food and feed contamination at the source, e.g. by reducing environmental pollution, (ii) applying appropriate technology control measure(s) in food and feed production, manufacture, processing, preparation, treatment, packing, packaging, transport or holding, and (iii) applying measures aimed at decontamination of contaminated feed or food and measures to prevent contaminated feed or food to be marketed for consumption”. Regarding biological contamination, most regulations demand the absence of pathogens in feed and food, setting standards for the sampling and analytical procedures.

Likewise, most countries lay down rules for agro-industrial wastes and wastewater management, in order to ensure an adequate level of safety and protection of public health. Typically, WaW are categorized based on their risk potential. Some WaW are not allowed to be applied to crops intended for human consumptions, e.g. municipal source WaW, while some highly hazardous WaW, e.g. hospital waste, are not permitted to be used for agriculture and must be disposed after appropriate treatment, including incineration. Other categories (including agro-industrial wastes and wastewater) might be used for the production of various commodities (such as organic fertilizers etc.) after appropriate treatment, such as composting or AD (see for example the EU REGULATION (EC) No 1069/2009). For most terrestrial plants transfer of contaminants from land applied waste to plant occurs via roots which act to mitigate the movement of contaminants into the above ground edible parts of the plant, and while many contaminants may accumulate in plant tissues they are often below most relevant risk thresholds (Chiou, 2003). Inadvertent transfer, e.g. via splashing during rain of irrigation events, is less likely to occur in massive doses (Heaton and Jones, 2008).

However, microalgae and duckweed are aquatic organisms, and unlike terrestrial plants they come in direct contact with any suspended or dissolved contaminants and therefore are more likely to be contaminated. Although there is an increasing interest to use agro-industrial WaW for biotechnological applications, like microalgal or duckweed cultivation, there are no known regulations or standards addressing the contamination risk of using WaW in such systems.

## 7. Conclusions

In many countries, agro-industrial WaW are legislatively categorized according to their origin (animal by-products, food waste etc.)

**Table 8**  
Summary of the potential for contamination of microalgae and duckweed cultivated with anaerobic digestates.

Contaminant	Comments
HMs	HMs are not removed during anaerobic digestion. However, most HMs are attached to the solid fraction after solid/liquid separation thus decreasing their amount available to microalgae and duckweed cultivated in the liquid fraction. The necessary dilution of digestates to prepare the cultivation medium, further decreases HMs concentration. There is a high potential of complexation and precipitation when pH of the medium reaches relative alkaline pH (> 8.5). The contamination risk with HMs might be low.
Pathogens	Most pathogens are removed during anaerobic digestion and especially under thermophilic conditions. However, there are some spore forming bacteria ( <i>Clostridium</i> or <i>Bacillus</i> ) that are resistant to thermophilic anaerobic digestion. A combination of different technologies might have higher removal efficiencies. Solid/liquid separation do not remove pathogens from the liquid fraction. The necessary dilution of digestates to prepare the cultivation medium, further decreases the abundance of pathogens and there is a high potential that pathogens are further inactivated during cultivation. The contamination risk with pathogens might be mainly low, but variable, and not always negligible.
Xenobiotics	<b>Hormones:</b> Low degradability under anaerobic digestion; after solid/liquid separation most of hormones are retained in the solid fraction. The necessary dilution of digestates to prepare the cultivation medium, further decreases the concentration of hormones. During cultivation of microalgae and duckweed estrogens are transformed, but the estrogenic activity of the degradation products might not be significantly decreased, or might even increase. A fraction of hormones is adsorbed on cell surfaces; thus the contamination potential risk is variable, but estimated as low to moderate. <b>Antibiotics:</b> degradation of antibiotics under anaerobic digestion varies significantly. Solid bound antibiotics are more persistent than the soluble fractions. The necessary dilution of digestates to prepare the cultivation medium, further decreases concentration of antibiotics and there is a high potential that antibiotics are further inactivated during the cultivation. Microalgae and duckweed might adsorb a fraction of the antibiotics. The contamination potential risk is low to moderate. <b>Parasiticides, mycotoxins and dioxins:</b> Their degradation under anaerobic digestion varies significantly. Some are complete, while other are negligibly degraded. During anaerobic digestion some dioxins might be newly formed. During cultivation some of the compounds might be degraded. Little data exists to assess the potential contamination risk.

which is then linked to their level of health risk and which in turn informs the regulatory approaches to their further usage or disposal. Human waste and animal by-products are more strictly regulated due to their more obvious health risks. Thus, many countries have developed their own regulations regarding the presence of various contaminants in feed or food, by setting Acceptable Daily Intakes, Acute Reference Doses, or recommendations for Maximum Residue Levels, and often impose strict safety monitoring processes. These regulations are of particular relevance for the initiation of a discussion on whether processed agro-industrial WaW might and ought to be used for cultivating microalgae or duckweed for feed or food production. In Table 8 the various potential contaminants, their removal during AD and cultivation along with an estimation of contamination risks are summarized. Contamination of biomass could occur either due to the adsorption of contaminants on surface or due to the intracellular accumulation. Surface adsorbed contaminants could be desorbed thus decontaminating the biomass, while the intracellularly accumulated contaminants cannot be removed, unless they are biodegraded into harmless by-products. It is important to identify strategies and devise methods to either reduce the concentration of contaminants in the growth media, e.g. by pre-treating of WaW streams, or to manipulate the cultivation conditions in order to inhibit uptake of contaminants by microalgae and duckweed, thus mitigating biomass contamination. Nevertheless, it is rather obvious that the topic of biomass contamination deserves further investigation and offers a wide scope for research.

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