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# Recent applications of biological technologies for decontaminating hormones in livestock waste and wastewater

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## Abstract

Large quantities of natural and synthetic hormones contained in livestock waste and wastewater (LWW) can cause serious problems in our environment. Composting and anaerobic digestion cannot remove hormones efficiently, so they should be modified to enhance the treatment processes. In addition, constructed wetlands show decent rates for removal of hormones. Advanced technologies such as membrane biological reactors and microalgae-based systems efficiently eliminate hormones from LWW. However, more practical studies are needed to investigate their actual performances. The categories, degradation mechanisms, and enzymes of hormone-degrading microorganisms are presented, and related hormone-degrading microorganism-based technologies are introduced. Finally, composting, anaerobic digestion, constructed wetlands, membrane biological reactors, and microalgae-based systems are compared in terms of their applicability in LWW treatment.

## Addresses

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## Keywords

Hormones, Composting, Anaerobic digestion, Membrane biological reactors, Microalgae-based systems, Constructed wetlands.

## Introduction

Natural and synthetic hormones are typical endocrine-disrupting compounds that are responsible for the reproductive disruption in marine organisms and

aberrant development of the gonads in animals and humans [1,2]. These chemicals are largely discharged from livestock waste (e.g. manures and urine) and wastewater (LWW). The total daily excretion of estrogens by cows has been estimated to be 145.2–179.3 µg/d, mainly in manure (92%), and by swines 42.6–219.3 µg/d, mainly in urine (98–99%) [3]. Consequently, high levels of estrogens were detected in livestock wastewater and the concentrations of estrone (E1), 17β-estradiol (E2), and 17α-ethinylestradiol (EE2) were 17.2–4728 ng/L, 8–542 ng/L, and 182–357 ng/L, respectively [4]. Owing to the low efficiency for hormone removal by conventional treatment processes, the residual hormones in LWW can subsequently contaminate the receiving rivers and soils, thereby posing a significant threat to the environment [5]. Therefore, alternative technologies that are suitable for rural areas are needed to eliminate threats from hormones in LWW.

Composting and anaerobic digestion (AD) are conventional technologies used to treat LWW, and they exhibit moderate success in hormone removal; thus, many approaches have been developed to improve efficiency for removal [6]. Constructed wetlands (CWs) can remove many kinds of hormones effectively [7]. In the past decade, several advanced biological technologies such as membrane biological reactors (MBRs) [5] and microalgae-based systems (MBSs) [8] were proven to have better hormone removal efficiencies than composting and AD. In addition, the technology based on hormone-degrading microorganisms (HDMs) has also been developed, and this may make a significant contribution to hormone control in LWW [9].

The objective of this study is therefore to introduce the major biological processes that eradicate hormones from LWW. Improvements in composting and AD, the performance of CWs, and the applications of several novel technologies, including MBRs, MBSs, and HDM-based processes, will be presented. Typically, the recently published literature will be highlighted, especially work published within the last two years. Finally, five bioprocesses will be compared with respect to five factors to be considered in selecting the proper technology for LWW treatment.

## Conventional treatment processes

### Composting

Composting is an effective way to treat livestock manure and produce organic fertilizers [10]. The degradation of hormones during manure composting has been amply studied, and both estrogens and androgens can be removed with efficiencies ranging from 40.4% to 90% [1,11,12]. However, the fate of hormones during composting can be affected by many factors including composting conditions, presence of other pharmaceuticals, and hormone molecular forms (free or conjugated) [1], and the removal efficiency (RE) is always limited by long operation times, and hormone residues are left in composted products [12]. Therefore, advanced composting processes are proposed to promote hormone removal in manure. In the last two decades, biochar has become a useful additive for manure composting by improving composting performance, enhancing microbial activities, and immobilizing hydrophobic organic pollutants [1,13]. Several studies have reported acceptable efficiencies (e.g. 71.0%–88.1%) for removal of estrogens from compost [14] and soil environments by biochar [15–17]. Furthermore, humic acid has also been confirmed to be an efficient material for removing hormones [1,18]. However, its practical use in composting still needs to be verified.

### Anaerobic digestion

From a sustainability perspective, AD has already become a promising method for LWW treatment. It was reported that many of the hormones can be degraded during waste or wastewater AD processes mainly by two mechanisms, biodegradation and biosorption [19]. However, according to work published thus far, the efficiency for removal of hormones through AD largely depends on the hormone category. Researchers evaluated the elimination of various progestogens from cattle manure and swine wastewater, and significant reductions in contaminant concentrations were observed in both AD systems [20,21]. Nevertheless, one study showed that the performance of AD in treatments of several hormones (i.e. estrone [E1], estradiol [E2], estriol [E3], and ethinylestradiol [EE2]) was limited, and total efficiencies for removal of estrogen from animal manure were only 14.7–21.8% [11]. In one case, the total estrogenicity of dairy manure was even increased by 23% after the AD process [22]. Therefore, conventional AD is not always an effective method for remediating hormone contamination.

Recently, several advanced anaerobic digestion processes were developed to enhance contaminant removal from manure [6]. Hamid and Eskicioglu [23] found that microwave pretreatment at higher temperatures (120 and 160 °C) for mesophilic AD led to total hormone reduction efficiencies that were approximately 50% higher than that of the control. In another study, the removal

rates for estrogen after treatments with an up-flow anaerobic sludge blanket combined with a step-fed sequencing batch reactor reached almost 78% [24]. Recently, Louros et al. [25] investigated the operating modes of up-flow anaerobic sludge blanket reactors and their influence on contaminant removal. Their results suggested that the intermittent operation led to improvements in the total removal of estrogens (>95% for E1 and EE2) compared with continuous operation (49% for E1 and 39% for EE2).

### Constructed wetlands

CWs are environmentally friendly and natural treatment technologies that are known to be efficient in purifying domestic and livestock wastewater in rural areas [7,26]. They exhibit various advantages including low cost of operation and maintenance, absence of power consumption, noise and offensive odors, and considerable improvement in the effluent quality [27]. Many studies have reported that CW-based treatment processes are capable of removing different types of micropollutants [28,29], including steroidal hormones such as estrogens, progestogens, androgens, and glucocorticoids [26]. The major hormone removal mechanisms include biodegradation (aerobic or anaerobic), sorption, and plant uptake [26]. For instance, biodegradation mainly contributes to the removal of 17 $\beta$ -estradiol ( $\beta$ E2) and testosterone [30], whereas E1, levonorgestrel, and norethisterone are mostly removed via absorption by certain plant species [31,32]. Table S1 summarizes the elimination of hormones in rural wastewaters effected by different types of full-scale CWs in the last decade. Recent studies found that the REs of modified CWs (i.e. combination, artificial aeration, and using baffles) ranged between 90.2% and 97.4% [33–35]. Many studies have been focused on the effects of design and operation factors on the performance of CWs [26]. Based on these findings, a group of researchers successfully established a model with which to predict the hormone REs of CWs and found that area, hydraulic loading rate, organic loading rate, and hydraulic retention time were the key factors determining model performance [36]. Furthermore, in several subsequent publications, they developed a novel decision tree framework to support decisions about the design, operation, and performance of CWs for the removal of 11 steroidal hormones [37].

## Advanced treatment processes

### Membrane biological reactors

Membrane bioreactors (MBRs) have been applied in various livestock wastewater treatments designed to remove refractory organic pollutants [5,38]. This technology has many advantages, such as a small surface area footprint, flexibility in operation, high biomass diversity, and high effluent quality, and it is suitable for use in rural areas. It has been proven that MBRs can efficiently

remove steroids or hormones from wastewater, with the removal rates of more than 80% [39]. The excellent efficiencies for removal of hormones by MBRs are mainly attributed to membrane components. MBRs can effectively retain high concentrations of activated sludge with enriched microbial assemblies [5], which may contain HDMs with acceptable bioactivities, such as ammonia-oxidizing bacteria [40] and nitrate-dependent anaerobic methane oxidation bacteria [41]. MBRs may effectively remove hormones from wastewater and also maintain stable performance with various environmental factors or operation parameters. Trinh and van den Akker [42] demonstrated that the total efficiencies for removal of testosterone, E1, androstosterone, and E2 in MBR (using microfiltration membrane modules) did not present significant differences between summer and winter, although the contribution ratio of biotransformation decreased slightly in winter. Researchers also reported that operational parameters such as flux rate (13–30 L/[m<sup>2</sup>·h]) and solid retention time (21 days vs 60 days) did not affect the hormone removal rates in MBRs with ultrafiltration and microfiltration membranes, respectively [43,44]. The previous studies further demonstrated that MBRs are quite effective and stable in treating wastewater containing harmful hormones. To further decrease the hormone concentrations in the MBR effluents, improvement measures have been applied. Recently, EE2 removal was evaluated in an MBR (with a microfiltration membrane) coupled with reverse osmosis or UV/H<sub>2</sub>O<sub>2</sub> as advanced oxidation process, and both hybrid processes exhibited >99% removal rates, whereas MBRs alone showed an 82–90% removal rate [45]. In addition, it has been reported that activated carbon (AC) (including powder AC [ $<50\ \mu\text{m}$  in size] and granular AC [100–2400  $\mu\text{m}$  in size]) coupled with MBRs achieved significantly higher efficiencies for hormone removal than MBRs alone [46].

### Microalgae-based systems

MBSs have been recognized as a novel and green technology for treating agricultural waste and wastewater; they can realize both nutrient recycling and removal of micropollutants [8,47]. MBSs eradicate hormones mainly via biodegradation [8], although photodegradation also occurs in some cases [47,48]. Researchers have already discovered various microalgae species that degrade hormones, and the related genera include *Chlamydomonas* [49], *Chlorella* [49,50], *Desmodesmus* [49], *Haematococcus* [50], *Microcystis* [51], *Scenedesmus* [50], and *Selenastrum*. Some of these species have been successfully used to eradicate estrogens in wastewaters, such as biogas slurries [52] and synthetic wastewater [53]. To enhance treatment performance, immobilization of microalgae in a matrix (e.g. beads) was developed, and the efficiency of E2 removal by this technology reached 85–99% [54]. To date, several laboratory- or pilot-scale MBSs have the ability to

efficiently remove typical steroid estrogens from different types of wastewater (Table S2). For instance, an algal-bacterial bioreactor combined with hydrothermal processing effectively treated the liquid portion of animal manure contaminated by three estrogens, and the removal rates for E1, E2, and E3 were ~95%, 94%, and 89%, respectively [55]. Nevertheless, it should be noted that too high concentrations of hormones in the influent of MBSs may hinder efficient functioning of the system and jeopardize the safety of the algal products (contaminant residues). Therefore, pretreatment processes such as AD are often used to remove some of the organic micropollutants before introduction into the MBSs [47,56].

### Hormone-degrading microorganisms and enzymes

As mentioned previously, several biological processes play important roles in removing hormones from LWW; thus, it is important to evaluate the properties of microorganisms and their hormone-degradation capabilities. Recent reviews have comprehensively summarized the categories of HDMs, their pathways for degradation of certain hormones and enzymes, and related genes involved [57–59]. To date, the widely accepted hormone biodegradation mechanisms are (i) metabolism in which hormones serve as carbon sources for HDMs and (ii) cometabolism in which enzymes produced by HDMs degrade hormones by using other energy sources. For instance, *Novosphingobium* sp. E2S efficiently degrades E2 and uses it to support growth [60]. It is believed that nitrifying bacteria are capable of cometabolizing steroids with the help of the ammonium monooxygenase enzyme [9]. Furthermore, many enzymes with various functions supporting hormone degradation have been discovered, such as dehydrogenase, hydroxylase, cytochrome P450, dioxygenase, and laccase [9].

Based on currently isolated HDMs and hormone-degrading enzymes, several hormone removal technologies using immobilized microbial systems have been developed [59]. Liu immobilized *Novosphingobium* sp. ARI-1 [61] and *Rhodococcus* sp. JX-2 [62] in calcium alginate, and the immobilized microorganisms both presented excellent efficiencies for removal of estrogen from cow dung. In a recent study, two HDMs (*Rhodococcus zopfii* and *Pseudomonas putida* F1) were encapsulated in small bioreactor platform capsules, and this approach might lead to practical use in wastewater treatment [63]. In addition to microbes, the use of immobilized enzymes (e.g. laccases) constitutes another effective and cost-effective technology for realizing practical decontamination of estrogen with improved catalytic stability, reusability, reduction of product inhibition, and ease of product separation [3].

Table 1

## Comparison of five biological technologies in terms of LWW treatment with hormones.

Biological technologies	Hormone RE	Energy consumption	Risk of secondary contamination	Nutrient recycling	Suitability for use in rural areas	Improvement approaches for hormone removal
Composting	40.4%–90% <sup>a</sup>	~16 kWh/t <sup>f</sup> [64]	Low	Producing fertilizer	Suitable	Applying additives (BC, HDMs, and HA) Pretreatment (pasteurization and microwave), post-treatment (soil infiltration and SFSBR), and mode switching
AD	14.7–21.8% <sup>b</sup>	30–50 kWh/t [65]	Moderate	Producing biogas and fertilizer (digestate)	Suitable	
CWs	9.0%–100% <sup>c</sup>	Nearly zero	Moderate	No	Suitable	/
MBRs	80%–99% <sup>d</sup>	0.6–2.3 kWh/m <sup>3g</sup> [66]	Unclear	No	Undetermined	/
MBSs	40%–100% <sup>e</sup>	Cultivation: 0.004–1.321 kWh/m <sup>3</sup> ; Harvesting: 0.0003–2.15 kWh/m <sup>3</sup> [67]	Unclear	Generating products with high values	Moderately suitable	Pretreatment (AD)

AD, anaerobic digestion; BC, biochar; CW, constructed wetland; HA, humic acid; HDM, hormone-degrading microorganism; LWW, livestock waste and wastewater; MBR, Membrane biological reactor; MBS, microalgae-based system; RE, removal efficiency; SFSBR, step-fed sequencing batch reactor.

Notes:

<sup>a</sup> The REs belong to conventional composting without any improvement approaches as shown in Section [Composting](#).

<sup>b</sup> The REs belong to conventional AD without any improvement approaches as shown in Section [Anaerobic digestion](#).

<sup>c</sup> The REs are concluded from the references shown in [Table S1](#).

<sup>d</sup> The REs are concluded from the references shown in Section [Membrane biological reactors](#).

<sup>e</sup> The REs are concluded from the references shown in [Table S2](#).

<sup>f</sup> The unit of 'kWh/t' is used to quantify the energy consumption to treat livestock waste (solid form).

<sup>g</sup> The unit of 'kWh/m<sup>3</sup>' is used to quantify the energy consumption to treat livestock wastewater (liquid form).

## Comparison of five bioprocesses

LWW is characterized by high concentrations of organic pollutants, heavy metals, and various micropollutants. Therefore, both decontamination and resource recycling should be taken into account to evaluate the applicability of technologies for LWW treatment and especially for hormone control. Specifically, five aspects (see Table 1) should be considered which are presented as follows:

- (1) Hormone RE: The performances of composting and AD for hormone removal are unstable or even unsatisfactory in most cases, especially for AD (only 14.7%–21.8%). To increase hormone REs, many modified processes such as enhanced composting and advanced anaerobic digestion were developed, as described in Sections 2.1 and 2.2. These improved technologies compensate for the drawbacks and maintain composting and AD as the most reliable technologies for LWW treatment. Unlike the conventional composting and AD, CWs, MBRs, and MBSs can effectively remove hormones from LWW.
- (2) Energy consumption: The energy consumed in composting was around 16 kWh/t (including compost operation, leachate, and odor gas treatment) [64], which is lower than the energy consumption of the AD process (30–50 kWh/t) [65]. CWs are vegetation-based technologies which consume limited energy during their operation compared with other technologies. MBRs have high energy consumption (0.6–2.3 kWh/m<sup>3</sup>) owing to the membrane component used in this technology [66]. MBSs are also energy-consuming technologies, and the energy consumption of algae cultivation and harvesting is 0.004–1.321 kWh/m<sup>3</sup> and 0.0003–2.15 kWh/m<sup>3</sup>, respectively [67].
- (3) Risk of secondary contamination: The estrogenicity of LWW may be enhanced by conventional AD treatment owing to the transformation of  $\alpha$ E2 (with lower estrogenicity) into E1 (with higher estrogenicity) under anaerobic conditions [22]. Therefore, LWW treated by AD may contain hormone residues that can pose adverse risks to the environment. Conversely, the composting process is operated under oxic conditions, so that the possibility of transformation of E2 into E1 can be speculated to be limited [9]. CWs can efficiently remove estrogens, whereas, secondary pollution of groundwater may occur through the leaching of CWs [5]. In addition, the risks of secondary contamination in LWW treated by MBRs and MBSs are still not clear and need to be studied in the future.
- (4) Nutrient recycling: The products of composting and AD can serve as fertilizers and biogas, which would realize nutrient and energy recovery from LWW. Besides, MBSs can generate products with high values (e.g. biofuel).

- (5) Suitability for use in rural areas: Composting and AD have long been applied for livestock waste treatment, so they are quite suitable in rural areas. CWs are widely used to treat livestock wastewater in rural areas because they are inexpensive and simpler to operate than other technologies. Although they have a large surface area footprint, this is not a problem in rural areas. Owing to the high cost in construction and operation, MBRs may not be economically viable in undeveloped areas. Nevertheless, the compact plant structure may provide an advantage for small livestock farms. Therefore, the applicability of MBRs in rural areas has not been determined, and this needs to be studied in the future. The main drawback of MBSs is their low tolerance for the organic load of LWW. As a result, pretreatment (e.g. AD) is needed to lower the concentrations of organic pollutants in the influent, when MBSs are intended to be used in rural areas.

## Conclusions

Biological processes are major technologies for removal of hormones from LWW. Composting and AD only partially remove hormones but can be modified to enhance the treatment performance. CWs have been successfully applied for removing hormones with decent REs. Advanced biological technologies such as MBRs and MBSs have been shown to have effective removal capabilities toward hormones. However, these technologies are not sufficiently mature to allow widespread application in livestock farms. Therefore, more pilot-scale and full-scale experiments are needed in the future. Furthermore, knowledge of HDMs, including their categories, degradation mechanisms, and active enzymes, has been actively pursued, and related HDM-based technologies have been introduced. Finally, a comparison of five biological technologies was presented herein to facilitate evaluation of their utility for treatment of LWW.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.coesh.2021.100307>.

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