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Sweet sorghum for phytoremediation and bioethanol production

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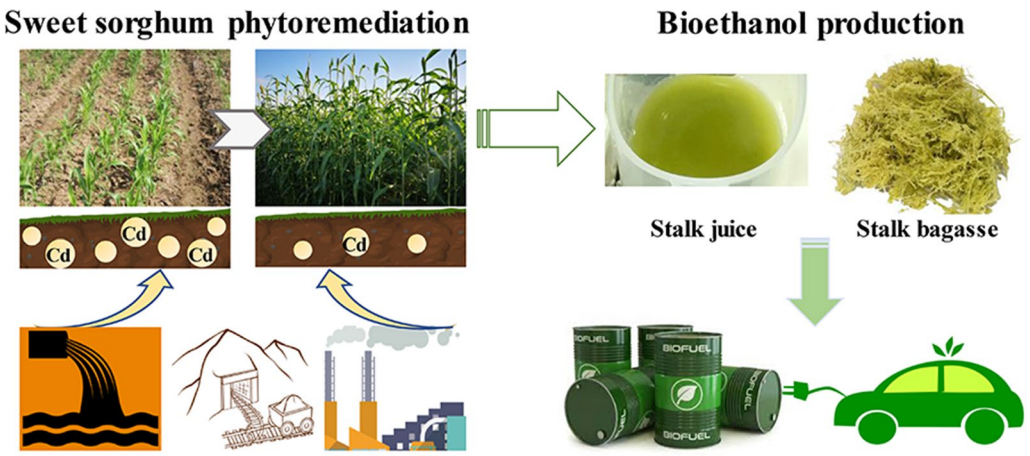
Abstract

As an energy crop, sweet sorghum (*Sorghum bicolor* (L.) Moench) receives increasing attention for phytoremediation and biofuels production due to its good stress tolerance and high biomass with low input requirements. Sweet sorghum possesses wide adaptability, which also has high tolerances to poor soil conditions and drought. Its rapid growth with the large storage of fermentable saccharides in the stalks offers considerable scope for bioethanol production. Additionally, sweet sorghum has heavy metal tolerance and the ability to remove cadmium (Cd) in particular. Therefore, sweet sorghum has great potential to build a sustainable phytoremediation system for Cd-polluted soil remediation and simultaneous ethanol production. To implement this strategy, further efforts are in demand for sweet sorghum in terms of screening superior varieties, improving phytoremediation capacity, and efficient bioethanol production. In this review, current research advances of sweet sorghum including agronomic requirements, phytoremediation of Cd pollution, bioethanol production, and breeding are discussed. Furthermore, crucial problems for future utilization of sweet sorghum stalks after phytoremediation are combed.

Keywords: Sweet sorghum, Bioenergy crop, Phytoremediation, Cadmium, Bioethanol, Pretreatment

Graphical Abstract



1 Introduction

As a consequence of contamination from increasing anthropogenic activities including mining, metal processing and smelting, industrial emissions, overuse of

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chemical products such as pesticides and fertilizers, and sewage irrigation, heavy metal (HM) pollution has become an increasingly serious problem worldwide [1, 2]. Various heavy metal(loids)s have contaminated more than 5×10^6 locus globally covering 2×10^9 hectares of land with soils [3]. Cadmium (Cd) is gaining attention as one of the most toxic HMs. According to the China Ecological Environment Status Bulletin in 2020, Cd is the primary HM contaminant in agricultural land [4]. Cd contamination modifies soil properties and induces soil degradation, resulting in the retardation of plant growth and substantial reductions in crop yield [5, 6]. Worse still, Cd is non-biodegradable and can thus accumulate in the environment and subsequently contaminate the food chain via plant uptake, generating health risks such as teratogenic, mutagenic, and carcinogenic effects [7, 8]. Therefore, there is an urgent need for remediation of Cd-contaminated soil.

Various techniques for the remediation of HM contaminated soil have been reported. Most physical remediation techniques (e.g., soil replacement, thermal treatment, and electrokinetic remediation) and chemical remediation techniques (e.g., soil washing and flushing, chemical stabilization/immobilization, and solidification) have limitations, including high costs, operational complexity, low efficiency, and irreversible changes to soil properties [9, 10]. Furthermore, chemical methods may generate groundwater pollution and increase the risk of secondary pollution [11]. In the mid-90 s, phytoremediation was proposed to rely on plants for the decontamination of polluted environment (phytovolatilization and phytoextraction) or stabilizing pollutant into harmless status (phytostabilization/phytoimmobilization) [10, 12]. Since this plant-based technology not only easy to operate but also economically viable, it is suitable for large and diffusely areas [7, 13]. Although hyperaccumulators have high HM bioaccumulation rates, their slow growth and low biomass are not ideal. On the contrary, high biomass plants offer good potential for the phytoremediation of soils, which can compensate for their low metal concentrations with high-yielding ability [14, 15].

For HM contaminated arable land, growing suitable metal-tolerant energy crops to remove HM while harvesting valuable energy products can be a viable economic alternative of land management strategy to food or feed production [12, 16, 17]. Furthermore, cultivation of energy crops on contaminated land would address the food-versus-fuel issue favorably. With this in mind, researchers have examined the HM tolerance of sweet sorghum and evaluated its HM absorption capacity [12, 18, 19]. Especially, recent studies have confirmed that some sweet sorghum varieties could achieve effective Cd removal while producing large biomass in Cd-enriched farmland [20–22]. Therefore, sweet sorghum is considered as a promising candidate for bridging phytoremediation and bioethanol production and thus prevent HM from entering the food chain.

Throughout the world, over 80% of energy sources still come from fossil fuels. However, the increasing depletion of fossil fuel and concerns associated environment has shifted worldwide attention to cleaner energy. Renewable fuel production from biomass has been considered a way to reduce the overdependence on fossil fuels [23–25]. Currently, as a biodegradable and renewable resource, bioethanol is the most consumable biofuel in the transportation sector, and has a brilliant future in easing the global energy crisis as well as the environmental pressure [26]. As shown in Fig. 1, global production of bioethanol has reached 2.9×10^{10} gallons annually [27]. However, the first generation (1G) bioethanol production from starch- and sugar-based stocks endanger food security; the second generation (2G) bioethanol production from lignocellulose materials is still questionable in terms of technological challenge and economic feasibility [25, 28, 29].

As an ideal energy crop for biofuel production, sweet sorghum is fast-growing and high biomass-producing C_4 annual grass (refers to the plants using the C4 photosynthetic pathway which converts CO_2 into 4-carbon intermediate), with outstanding adaptability to harsh conditions like drought, heat, waterlogging, and salinity [26]. It is widely cultivated in subtropical, tropical, and semi-arid tropical regions. The total aboveground fresh biomass yields range from 55 to 150 t/ha [30]. Compared with grain sorghum, sweet sorghum varieties are much taller and produce significantly higher biomass yields, with the fleshier and juicier stems but smaller seed heads

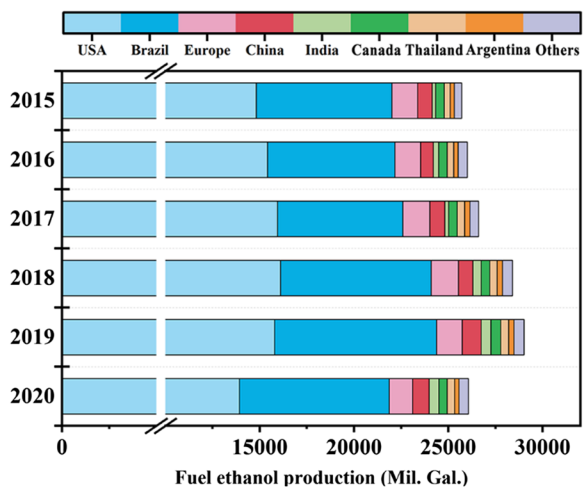

Fig. 1 Annual world fuel ethanol production from 2015 to 2020 [27]

[31]. Some sweet sorghum lines can yield 78% of the total plant biomass in juice, with juice Brix of 15–23%. The soluble fermentable sugars in the juice are comprised of 6–21% fructose, 9–33% glucose, and 53–85% sucrose [32, 33]. According to the previous report, dried sweet sorghum stalks (SSS) contained 50.7% soluble sugars, 19.6% cellulose, 15.2% hemicelluloses, and 3.2% acid insoluble lignin [34]. Due to the high production of both fermentable saccharides and lignocellulose, sweet sorghum is particularly suitable for producing various biofuels (e.g., biodiesel, bioethanol, biohydrogen, and biogas) and bio-based products (e.g., acetone, biobutanol, lactic acid, bacterial cellulose, and reinforcement additives for geopolymers) [35–40]. Especially due to the high soluble sugar contents, the bioethanol obtained from sweet sorghum could be taken as a 1.5 generation biofuel [41]. In contrast to other major sugar crops such as sugarcane and sugar beet, the demand for energy to produce raw sorghum juice for ethanol production is lower [42].

Phytoremediation of Cd-contaminated land by sweet sorghum would provide relatively positive remediation results and generate large amounts of biomass for bioethanol production with low input. Comparing with traditional physical and chemical remediation methods, this strategy is more environmentally friendly. And the utilization of sweet sorghum for bioenergy is more economically efficient than hyperaccumulators. Enabling this integrated strategy will be strongly conducive to improve the environmental and economic benefits of ecological restoration. Developing the comprehensive concept of phytoremediation combined with biorefinery will further establish guidance for remediation of other HM contaminated areas such as chromium (Cr). Due to the extensive use of Cr-containing tanning agents in the leather-based industries and the lack of appropriate disposal strategies of tanning sludge, the threats of Cr pollution from tannery to the surrounding environment should not be underestimated [43, 44]. Similarly, the selection of suitable energy crops for Cr phytoremediation may lead to a more sustainable and applicable approach.

This study provides an overview of researches on sweet sorghum relating to agronomic requirements, phytoremediation of Cd pollution, bioethanol production, and breeding. The characteristics of sweet sorghum in Cd phytoremediation are specifically discussed. The production of bioethanol from SSS is systematically elucidated. Then, targeted and comprehensive breeding aim is proposed. Finally, it critically assessed the potential and challenge for utilization of stalks after phytoremediation. Based on the significance of soil remediation, this paper is expected to contribute to the realization of sweet sorghum phytoremediation and simultaneous bioethanol production.

2 Characteristics and growth conditions

2.1 Characteristics

Sweet sorghum (*Sorghum bicolor* (L.) Moench) belongs to the grass family Poaceae, tribe *Andropogoneae*, and subtribe *Sorghinae*, originated in Africa. The genus *Sorghum* consists of those generally recognized as sorghum and some of their closer relatives, which is a group of plants with phenotypic, genetic, and geographic diversities. The enormous variation in the genus is divided into 22 species classified as five sections [42, 45, 46]. The term sweet sorghum is applied to distinguish those special genotypes with high accumulation of soluble sugars in the stem or sap [47]. At maturity, sweet sorghum can grow to a height of 250–580 cm, with an elliptical or round head as well as wide flat leaves. The stems are resembling those of maize, nearly oval with groove. The root system of sweet sorghum is fibrous with profuse branching. Under a feasible environment, the strong adventitious roots can be produced by above-ground nodes that help anchor the plant to reduce lodging [30, 33]. The C_4 photosynthesis contributes to higher nitrogen and water use efficiency as well as overall robustness of sweet sorghum, enabling it to better survival in the dry regions with higher light intensity/temperatures [31].

The traits of sweet sorghum are particularly favorable as a biofuel feedstock, such as short duration (approximately 120 days), good tolerance of abiotic and biotic stress, high photosynthetic efficiency, fewer input requirements, as well as low cost of cultivation [31, 47, 48]. SSS is the most essential part for bioethanol production, accounting for about 70% of the total aboveground dry weight. Yields of soluble and structural carbohydrates in SSS depend on their varieties, growing environment, and harvest time [26, 49]. Additionally, Appiah-Nkansah et al. [32] summarized the characteristics of sweet sorghum suitable for bioethanol production: (1) high biomass yield; (2) thick and lodging-resistant stalks with juicy internodes; (3) high total soluble sugar content of juice; (4) high juice extraction rate; (5) a long period of industrial use; and (6) a range of sweet sorghum varieties with different maturity levels to extend the harvest season.

2.2 Agronomic requirements

Although native to the tropics, sweet sorghum adapts well to temperate regions. It can be cultivated between 45°N and 45°S latitude, at elevations between mean sea level and 1500 m. Sweet sorghum is more heat tolerant than many other grain crops, with an optimum growth temperature of 32–34 °C. The minimum temperature for germination is 7–10 °C, and for growth is 15 °C [30, 47]. Under suitable climatic conditions (low latitudes with more frost-free periods), sweet sorghum can ratoon after

the main crop harvest, allowing for two cropping seasons in eight months [50].

Generally, sorghum can be cultivated successfully in multifarious soil conditions, including organic soils, calcareous soils, medium loams, and heavy clays, and can tolerant a soil pH range of 5.5–8.5 [30]. The most productive soil for sweet sorghum cultivation is well-structured and well-drained black or red clay loam soils with pH ranging between 6.5 and 7.5 [32, 47]. It was found that the nodal roots of sweet sorghum were longer and stronger in loam soil than those in clay soil, which had more efficient nutrient and water uptake, leading to a higher yield of juice, sugar content, and bagasse [51]. Sweet sorghum has strong resistance to saline-alkaline soils, which could produce sufficient sap, total carbohydrates, and bioethanol in fields with soil salinity up to 3.2 dS/m even if with a 25–50% reduction in irrigation [52]. Although sweet sorghum is generally tolerant of low nutrient levels and poor soil conditions, the balanced fertilization is required for a productive crop and the content of fertilizers varies with the level of N, P, and K in the soil profile [32, 53, 54]. The previous research found that sweet sorghum needs only 36% of the fertilizer N demanded by corn to obtain similar ethanol yields [55]. Considering the biomass, sugar yields, and nutrient recoveries, Erickson et al. pointed that the optimal requirements for the long-term whole plant harvesting were 90 to 110 kg N/ha and 15 to 20 kg P/ha, respectively [56]. Besides, the K requirements are not low for high biomass yields of sweet sorghum, even though it only exhibits one critical K uptake stage, from elongation to anthesis. It has been reported that K uptake amounts ranged 109–300 kg/ha for the total aboveground dry weight of 13.2–35.2 t/ha [49, 57].

As known to be one of the most drought-tolerant crops, sorghum can remain dormant during drought and resume growth when appropriate conditions reappear. The large fibrous root system of sweet sorghum works effectively, which can extend up to a depth of 2 m, with approximately twice the capacity to absorb water from the soil than corn [30, 31]. Under drought stress, it was found that the water use efficiency in sweet sorghum increased by 20% while decreased by 5% in maize. Zegada-Lizarazu et al. [58] proposed that the better drought resistance in sweet sorghum attributes to its capacity to improve the water use efficiency, enhance root length density, and maintain high leaf water potential as well as physiological activity under drought stress. Sorghum will survive with less than 300 mm (rain and irrigation in total) of water over the 100-day growth period. Nonetheless, sufficient moisture is crucial for plant maximum production. Sweet sorghum requires 500–1000 mm of water to obtain well yields of 50 to 100 t/ha [47]. Besides, sweet sorghum is susceptible to sustained water logging. Thus, appropriate nutrient and water management are vital to optimizing biomass and sugar yields of sweet sorghum.

3 Phytoremediation of Cd pollution

3.1 Physiological and biochemical responses, and the Cd accumulation mechanisms under Cd stress

Previous studies have elucidated the physiological and biochemical responses of sweet sorghum under Cd stress in various aspects. Root is directly exposed to Cd thus the Cd stress could firstly reduce root activities, impede the absorption of water and nutrient, influence the cell cycle progression, and induce cell death in root tips of *S. bicolor* seedlings [59, 60]. As shown in Fig. 2a, the distribution of Cd-staining dye indicated that Cd primarily located in the meristematic zone. While the S-phase cells in the root tips labeled by EdU (ethynyl deoxyuridine) were reduced with increasing Cd concentration. Especially, the root activities showed negatively correlated with the Cd concentration at each growth stage [61]. During the seed germination and root growth of sweet sorghum, the Cd toxicity would impair the activities of hydrolyzing enzymes and the translocation of the hydrolyzed sugars from cotyledons to the growing embryonic axes, ultimately resulting in the reduction of germination and disruption of seedling growth [60]. For sweet sorghum seedlings, the chlorophyll (Chl) and carotenoid contents did not change significantly at low Cd exposure, but the decrease became increasingly severe with the increase of Cd stress. While the change of the shape of Chl *a* fluorescence transient, increase in Chl *a*/*b* ratio, reduction in stomatal conductance and transpiration rate, and obstructed electron transport in sorghum leaves have also been observed after Cd treatments. These demonstrated factors may together result in the decrease of photosynthetic activity of sorghum seedlings [61–64]. The ultrastructural alterations of sweet sorghum have been directly discovered under high Cd stress, including the impairment of the chloroplast structure (Fig. 2b) and the thickening of the cell walls of vascular bundle cells in leaves as well as xylem and phloem cells in roots [64].

The Cd-induced reactive oxygen species (ROS) could lead to oxidative damage in plants, including O^{2-}, OH^{-}, and H_2O_2. The oxidative stress to sweet sorghum under low Cd concentrations ($\leq$10 mg/kg) stress could stimulate antioxidant defence system to eliminate ROS. While high levels of Cd ($\geq$50 mg/kg) would reduce the activities of antioxidant enzymes in sweet sorghum plant such as peroxidases and glutathione transferase, and overcome their quenching capacity, simultaneously causing cell damage [62, 66]. The Cd stress could also alter the expression levels of auxin-related genes in the roots of sweet sorghum seedlings, thereby disturbing the homeostasis of auxin and ROS, resulting in the growth inhibition [59].

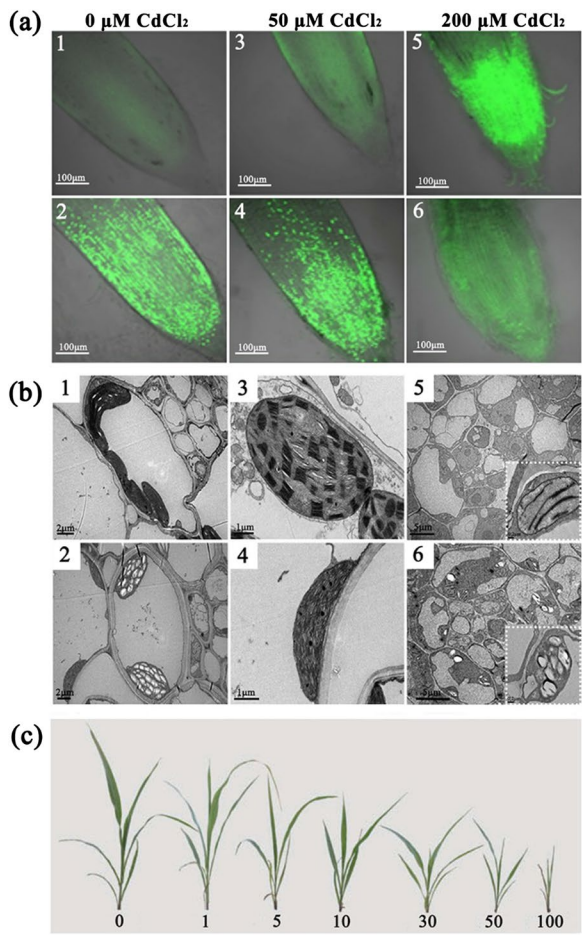

Fig. 2 Cd stress inhibits sweet sorghum plant growth. **a** Accumulation of Cd and Cd inhibited S-phase cell cycle progression in root tips. 1, 3, 5: Cd localization revealed by Leadmium Green AM staining in the root tips of *S. bicolor* seedlings exposed to 0, 50, and 200 μM $CdCl_2$ for 5 days. 2, 4, 6: Images generated by merging the confocal images of EdU-labeled cells with bright-field microscopy images. Adapted by permission from [59]. Springer Nature, Copyright (2017). **b** Transmission electron microscopic micrographs of chloroplast. Seedlings of sweet sorghum were treated with 0 (1, 3, 5) and 100 μM (2, 4, 6) Cd for 30 days. 1 & 2: Vascular bundle sheath cells in the Kranz anatomy of leaves. 3 & 4: The chloroplast of mesophyll cells. 5 & 6: The Kranz anatomy of caulicles. One separate chloroplast was illustrated in the bottom-right corner (scale bars = 0.2 μm). Reprinted by permission from [64]. Springer Nature, Copyright (2016). **c** Morphological responses to the increase of Cd concentration (0–100 mg/kg, the 51st day). Reprinted from [65], Taylor & Francis Ltd, (http://www.tandfonline.com), Copyright (2015)

Additionally, Cd^{2+} may compete with bivalent metal ions (such as Fe^{2+}, Zn^{2+}, and Mn^{2+}) for the transport binding sites and further interfere with the accumulation of micronutrients in sweet sorghum [67]. The inhibitory effect of Cd on sweet sorghum growth (Fig. 2c) determines that sweet sorghum is more adapted to soils with mild level of Cd contamination.

The molecular mechanisms of Cd uptake, translocation, and accumulation to sweet sorghum remain mostly unknown up to now. Feng et al. [67] have made great efforts to gain a preliminary understanding of these molecular mechanisms. Two sweet sorghum genotypes with contrasting Cd translocation factors were comparatively investigated (Accession No. PI 152873, with high-Cd accumulation; Accession No. PI 273969, with low-Cd accumulation). Not only did they differ greatly in the symplasmic Cd uptake by root, but the root anatomy structures also revealed differences in their endodermal apoplasmic barriers. Underlying these traits, many differentially expressed genes (DEGs) involved in cell wall metabolism and modification between these two genotypes were identified by transcriptome data, while DEGs encoding HM transporters were also examined. Besides, KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analysis showed over-representation of phenylpropanoid biosynthesis pathway both for Cd-responsive DEGs and DEGs, indicating the importance of this pathway in Cd response and the differential Cd accumulation of sweet sorghum. Recently, Jia et al. [68] further performed a comparative analysis of small RNAs, degradome, and transcriptome in these two differential sweet sorghum genotypes to reveal the regulatory mechanisms behind Cd accumulation. Potential MicroRNAs with their target genes involved in sweet sorghum response to Cd stress were identified. These MicroRNA targets may participate in cell wall construction, transmembrane transportation, cytoskeleton activity, and ROS homeostasis.

Combined with the analyses of morpho-physiological traits and molecular mechanisms, Feng et al. [67] finally constructed a diagram to illustrate the key processes affecting the Cd uptake and translocation in sweet sorghum plants as displayed in Fig. 3a. It was proposed that the high Cd accumulation may be mainly realized by the synergy of multiple processes including efficient root uptake (Fig. 3a step 1), less root cell wall binding (Fig. 3a step 2), weak endodermis apoplasmic barriers (Fig. 3a step 3), and efficient xylem loading (Fig. 3a step 4). Furthermore, another previous study by their research team [64] showed that the distribution of Cd entering sweet sorghum seedlings was not homogeneous in different tissues. The localization of Cd was investigated in situ by dithizone staining method. The images of tissue sections (Fig. 3b) showed that Cd was mostly centralized in the stele of roots while dispersed in the intercellular space of caulicles.

3.2 Cd Phytoremediation capacity

The experiments relating to Cd phytoremediation by sweet sorghum are collated within Table 1. In 2005,

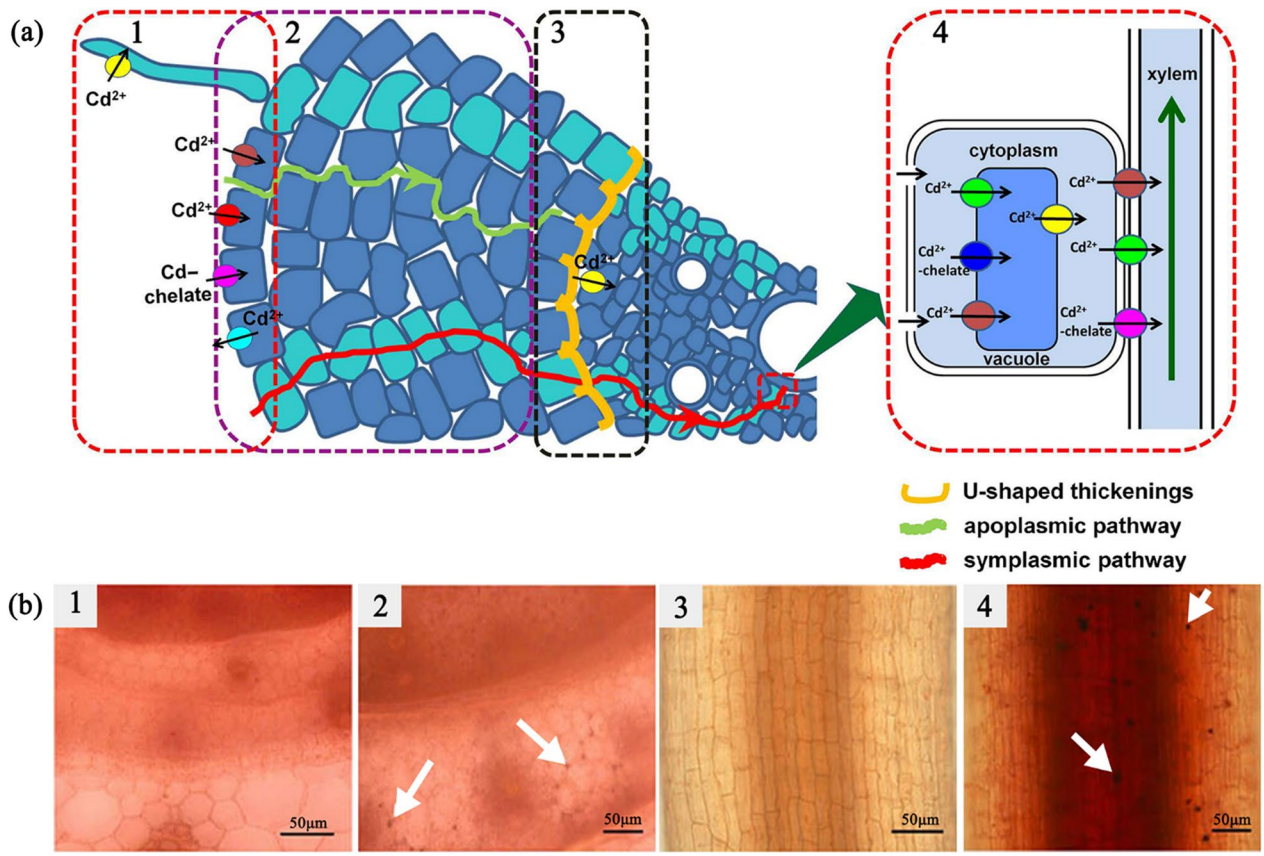

Fig. 3 The mechanisms of Cd accumulation in sweet sorghum plants and the localization of Cd in different tissues. **a** The main physiological processes involved in Cd uptake and translocation: 1, uptake of Cd from the external solution to root cells; 2, cell wall binding of Cd; 3, apoplastic barriers in the endodermis; 4, Cd translocation via xylem. Adapted from [67], John Wiley & Sons, Inc. Copyright (2017). **b** Cd-dithizone precipitates in caulicle (1, 2) and root (3, 4). Sweet sorghum seedlings were exposed to 0 (1, 3) or 100 μM (2, 4) Cd concentration for 3 weeks. Adapted by permission from [64]. Springer Nature, Copyright (2016)

Marchiol et al. [18] conducted the first in situ field trial to estimate the phytoremediation ability of sweet sorghum in an industrial site polluted by pyrite cinders (located at Torviscosa, Italy). The absence of nutrients in the native soil significantly impeded the growth of sweet sorghum and therefore their removal of Cd was negligible. After treatment with mineral fertilization and organic amendment, sorghum could produce adequate biomass and absorb total Cd content of 5.62 and 4.31 g/ha, respectively. Meanwhile, the highest removal efficiency of HMs in the soil by sweet sorghum was 0.030% of As, 0.056% of Cd, 0.024% of Co, 0.225% of Cu, 0.018% of Pb, and 0.082% of Zn, respectively. Afterwards, Zhuang et al. [19] established a field plot experiment using sweet sorghum for polymetallic paddy soil phytoremediation. In the field site seriously polluted by lead and zinc mining wastewaters (Lechang, China), sweet sorghum Keller could achieve the total removal of 52 g/ha for Cd after 120-day cultivation without any treatments. Besides, the removals of Zn and Cu (1.44 and 0.24 kg/ha, respectively) were also considerable. Another in situ phytoremediation experiment carried in industrially polluted regions near Plovdiv, Bulgaria also confirmed the synchronous accumulation of Pb, Cu, Zn and Cd in sweet sorghum [69]. Particularly, compared with other crops such as sunflower, maize, barley, and *Nicotiana tabacum*, sweet sorghum has the strongest Cd extraction in multiple HMs contaminated soil [19].

To explore the phytoremediation potential of sweet sorghums in soil with only Cd pollution, researchers further carried out targeted pot experiments. Yajin No.1 has been reported to have the highest Cd uptake of 2.47 mg/plant when the Cd concentration in the soil was 30 mg/kg, meanwhile the aerial biomass was 82.1 g/plant [65]. Wang et al. [70] grew sweet sorghum in the pots with acidic sandy loam soil (pH 6.1), and found that Nengsi 2# could absorb up to 2.70 mg Cd/plant under Cd stress of 15 mg/kg with the aboveground biomass of 36.1 g/plant. Similarly, a controlled plot experiment was performed to test the phytoremediation potential of sweet

Table 1 Experiments relating to the sweet sorghum phytoremediation

Species	Remediation scale	Growing conditions		Cultivation time	Aerial biomass dw	Cd concentrations (ppm, dw)	Cd uptake	References
		Type	Total Cd (ppm)					
-	Field trial	Native soil, 142 m^2	4.29	112 day	1.54–22.1 t/ha	Root 1.35–1.75 Shoot 0.20–0.26	0.31–5.62 g/ha	[18]
Keller, Mray, Rio	Field trial	Paddy soil, 288 m^2	4.9	120 day	18.7–25.8 t/ha		26–52 g/ha	[19]
Sugar sorghum	Field trial	Calcaric Alluvial soil, 25 m^2	2.5–26.2	Reaching ripeness	–	Root 1.1–7.5 Stem 0.14–0.33	–	[69]
-	Pot test	Vermiculite with Hoagland solution	50	10 week	0.94 g/plant	Root 88.8 Aerial part 13.7	–	[74]
Six hybrids	Hydroponics	Modified Hoagland solution	200 μM	28 day	–	Root 0.44–1.1 Stem 0.08–0.20	–	[62]
Yajin No.1	Pot test	–	1, 5, 10, 30, 50, 100	167 day	12.5–111.7 g/plant	Root 6.7–137.9 Shoot 6.3–30.6	0.48–2.47 mg/plant 52–271 g/ha	[65]
–	Field trial	–	4.52	120 day	37.6/55.1 t/ha	Root 3.4/3.9 Shoot 0.3/0.5	11/23 g/ha	[75]
M-81E	Hydroponics	Modified Hoagland solution	10, 50, 100 μM	30 day	–	Root 435–3565 Caulicle 27–68	–	[64]
	Pot test	Humus-vermiculite mixture	30	5 mon	–	Root 10 Stem 0.17–1.2	–	
Cowley, Nengsi 2#	Pot test	Acidic sandy loam soil	3, 15	100 day	30.2–63.9 g/plant	Root 9.7–46.1 Stem 6.2–70.6	0.49–2.70 mg/plant 50–280 g/ha	[70]
M64	Field control experiment	Sieved natural soil	2.3–33.6	167 day	126–194 g/plant	Root 5.4–24 Stalk 2.07–7.0	0.43–1.23 mg/plant	[71]
96 genotypes of sorghum	Hydroponics	Modified Hoagland solution	10 μM	2 week	–	Root 277.0–898.3 Shoot 19.0–202.4	6.1–25.8 μg/plant	[76]
107 sorghum accessions	Field trial	Alluvial soil, 100 m^2	2.24	2 mon	–	Leaf sheaths 5.8–58.6 Nodes and internodes 4.4–37.2	–	[77]
BL0602	Pot test	Quartz sand	50, 100 μM	15 day	–	Root 91, 135 Stem 27.5, 31.3	7.4, 10.1 mg/plant	[63]
L69, H18	Hydroponics	–	10 μM	2 week	–	Root 376, 904 Shoot 32, 208	–	[67]
Five hybrids	Field trial	Cropland soil, 21 m^2	2.0	5 mon	721–857 g/plant	Root 1.9–4.5 Stem 0.14–1.9	2.5–6.0 mg/plant	[20]
166 sorghum accessions	Field trial	Farmland soil	3.03, 2.80	Reaching maturity	95.6–1236 g/plant	Stem 0.5–16.5	0.12–1.6 mg/plant	[21]
Alto No.2	Pot test	Sieved paddy soil	1.22	90 day	128 g/pot	Root 5.25 Shoot 3.75	0.48 mg/pot	[73]
Six sorghum cultivars	Field trial	Farmland soil, 2 ha	0.25, 0.96	5 mon	20.4–27.9 t/ha	Stalk 1.3–9.2	19.6–148 g/ha	[22]
Dalishi	Hydroponics	Nutrient solution	5 μM	10 day	0.12–0.25 g/plant	Root 140–300 Stem + sheath 24.8–33	17.4–43.6 μg/plant	[72]

sorghum M64. It can be concluded that the Cd accumulation by M64 could reach up to 0.84 mg/plant with the dry weight of 171 g/plant when the soil Cd concentration was 18 mg/kg [71]. Soils with gradient Cd concentrations were used in these pot experiments. Although the sorghum biomass decreased with the increase of Cd stress, higher Cd level was more conducive to the Cd transfer from soil into the plants. Therefore, the total Cd removal quantity of potted sorghum was closely related to the soil Cd concentration, and the best remediation result was achieved under the intermediate conditions (15–30 mg/kg) of set Cd pollution.

Information gained in controlled pot conditions was limited, thus three field trials were conducted to verify the application perspective of sweet sorghum against the background of severe problem of Cd-polluted farmland in Hunan province, China. According to Yuan et al. [20], five species of hybrid sweet sorghum were planted in a cropland presenting a low contaminated soil with the Cd concentration of 2.0 mg/kg located at Chenzhou, Hunan. They found none of these hybrids showed obvious toxicity symptoms, while the hybrid 1794 had the highest Cd removal of 358 g/ha and dry mass of 760 g/plant. A screening test of 166 sorghum accessions (including 124 sweet sorghum) was carried out in a typical Cd-polluted agricultural field in Zhuzhou, Hunan by Liu et al. [21]. After the growing season of 2016 and 2017 (soil Cd concentration of 3.03 and 2.80 mg/kg), five optimal accessions were selected with the Cd accumulation ranging from 489 to 1174 μg/plant and biomass above 698 g/plant. Field trials on real planting scales of 2 ha and 1.22 ha in Hunan were performed in 2017 and 2018 by Xiao et al. [22]. In the farmland with low Cd pollution (Cd concentration of 0.96 and 0.25 mg/kg), six sorghum cultivars removed Cd 19.6–148 g/ha after one crop and produced dry aerial biomass in the range of 20.4–27.9 t/ha. Obviously, the Cd concentrations in farmland soils were much lower than those in pot test, and most sweet sorghum varieties could grow normally. But it was undeniable that the source of Cd pollution in the field was more complex and dynamic. Identifying sweet sorghums with high Cd absorption at low Cd pollution level and adapted to the local climate is significant for the promotion of practical application of phytoremediation.

3.3 Promoting Cd removal

The Cd removal capacity of sweet sorghum could be facilitated by appropriate agronomic practices, including soil fertility management, mobilizing agents, endophytic bacteria, and harvesting methods. Nitrogen fertilization is a common agricultural measure. High NH_4^+ containing fertilizer can decrease soil pH, leading to the increment in Cd uptake by plant. It is observed that the NH_4NO_3 and $(NH_4)_2SO_4$ treatments increased the biomass of sweet sorghum and minimally enhanced phytoextraction [19]. Through the hydroponics supplying nitrogen in the form of $Ca(NO_3)_2$, Bai et al. [72] further discovered that the Cd concentrations in sweet sorghum aboveground tissues displayed an inverted 'U' shape with increasing N levels under Cd stress. An optimum nitrate supply would increase both dry weight and Cd concentration, thereby resulting in higher efficiency of Cd phytoextraction. Organic mobilizing agents may mobilize HMs in soils and fertilize soils, moreover they are readily degradable. Applying the composited organic agents (citric acid + dissolved organic fertilizer) at heading stage achieved the maximum sorghum biomass and Cd bioaccumulation quantity, which were 3.8% and 48.8% higher than those of the control, respectively [73]. The plant-growth-promoting endophytes (PGPEs) with multiple HMs resistances originating from hyperaccumulator could facilitate the HM phytoremediation and biomass production of sweet sorghum. Sweet sorghums inoculation with the endophytic bacterial strain SLS18 significantly produced more biomass (increased by 38%) than the control groups in Cd-polluted pots, resulting in the increased Cd removal with little change of Cd concentration in plant [74]. In addition, the double harvesting method would also enhance the phytoextraction efficiency of sweet sorghum by increasing total biomass yield. It has been reported that the biomass and total Cd uptake of sweet sorghum under double harvesting increase by about 46.5% and 109% respectively compared to single harvesting [75]. The Cd accumulation in stalks was discovered increasing with maturity. Consequently, harvesting sweet sorghum after the dough stage would be beneficial to enhance the removal of Cd [22]. Although EDTA is considered as one of the most effective chelating agents, it did not show evident effects on Cd bioaccumulation for sweet sorghum when used as soil amendment [19].

3.4 Characteristics of sweet sorghum in Cd phytoremediation

According to the reported literature, sweet sorghum for phytoremediation of Cd pollution indicates the following five special features:

Firstly, the Cd tolerance and bioaccumulation in sorghum plants varied greatly amongst different sorghum genotypes. Considering the vast genetic diversity of sorghum, the investigations on diverse sorghum accessions under Cd stress have been carried for germplasm screening, including 96 sorghum genotypes in hydroponic condition [76], 107 cultivars in hydroponic cultures and under field conditions [77], and 166 sorghum accessions in field tests [21]. Several promising sorghum cultivars

were identified for restoring Cd contaminated areas, and Liu et al. [21] proposed that sorghums with different Cd accumulation properties could be applied for different end uses. More large-scale field experiments in different polluted environments are still needed to verify the phytoremediation capacity of sorghum varieties for tailored selection.

Secondly, sweet sorghum is not termed hyperaccumulator, but employed as high-biomass-producing non-hyperaccumulating plants for phytoremediation. So far, none of the sorghums has been reported meeting the Cd concentration threshold (100 mg/kg) in dry biomass of hyperaccumulator definition. High Cd pollution would seriously inhibit the growth of sweet sorghums, thus sweet sorghum phytoremediation is more suitable for moderate or low Cd pollution conditions ($\leq$30 mg/kg) [64, 65]. In low Cd-contaminated farmland and site near the abandoned mine, the abundant biomass reserves of sweet sorghums contributed to their Cd uptake, even making their Cd removal capacity quite competitive with many hyperaccumulators [20, 22].

Thirdly, while the Cd concentration in the root is obviously higher than those in the aerial parts for sweet sorghum, total Cd removal is mainly achieved by aerial parts especially stems for their high yields. As non-hyperaccumulator, the translocation factor of sweet sorghum (shoot-to-root ratio of Cd concentration) is < 1. Especially in short-term experiments cultivating sorghum seedlings under Cd stress, most of the absorbed Cd was still retained in the roots [63, 67, 76]. During the sorghum growth period, Cd is continuously transported from the root to the aerial parts in a low concentration. As illustrated in Fig. 4a, the results of tracking Cd levels in sweet sorghum at different growth stages showed that Cd concentrations in different tissues consistently exhibited an order of

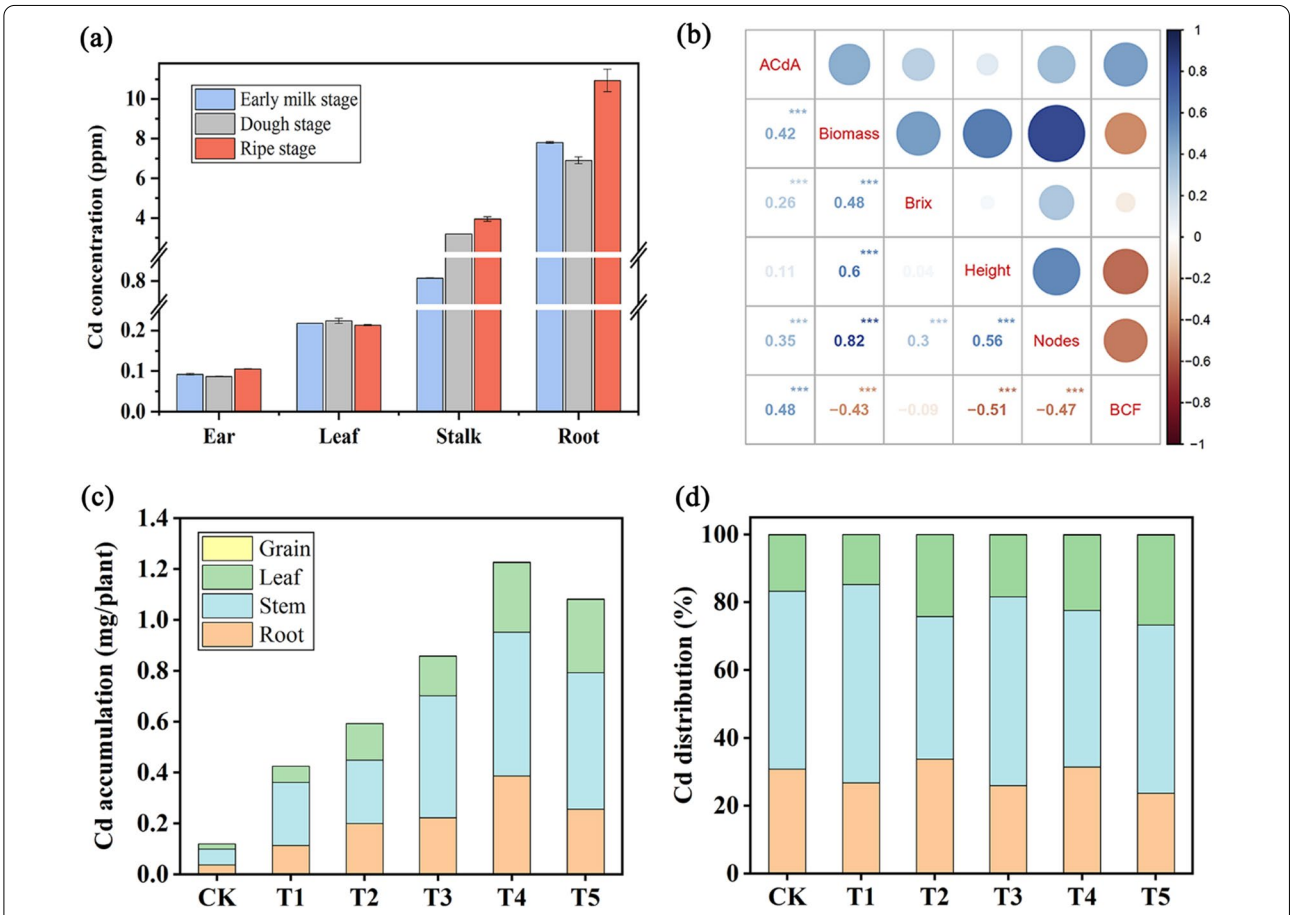

Fig. 4 Characteristics of sweet sorghum in Cd phytoremediation. **a** The Cd concentrations in different sorghum dried tissues. Adapted from [22], Elsevier B. V., Copyright (2021). **b** Pearson pairwise correlations among sorghum aboveground Cd accumulation (ACdA), the bioaccumulation factor (BCF), and bioenergy-related traits. Reprinted from [21], Elsevier B. V., Copyright (2020). The Cd accumulation (**c**) and distribution |(**d**) in the different organs of sweet sorghum (grown in soils with Cd concentrations of 0.25 (CK), 2.3 (T1), 5.9 (T2), 7.2 (T3), 18.1 (T4), and 33.6 (T5) for 167 days). Adapted from [71], with permission of the authors

root > stalk > leaf > ear. Specifically, it was observed that the Cd concentration in stalk increased substantially from the milk stage to the dough stage meanwhile in root decreased slightly [22]. Whereas, the proportion of root biomass in mature sweet sorghum is significantly small, causing the total Cd content in root lower than that in aerial parts [65, 71]. Even under different concentrations of Cd contamination, the Cd within mature sweet sorghum mainly accumulated in the stalk (Fig. 4c), and the normalized results showed that stalks accounted for the largest proportion of total Cd at 42–58% (Fig. 4d) [71]. This feature reminds that the germplasm screening for phytoremediation sweet sorghum should take sorghums in different growth periods into consideration, instead of restricting the screening scope to seedlings.

Fourthly, the aboveground Cd accumulation (ACdA) is strongly associated with bioenergy-related agronomic traits of sorghum. Based on the agronomic traits of the sorghum accessions grown in a typical Cd-polluted field, Liu et al. [21] performed a Pearson pairwise correlation analysis to explore the possible factors influencing Cd uptake in sorghum (as shown in Fig. 4b). It has been identified that the ACdA is positively correlated with the biomass, internode numbers, stem Brix, and plant height, which are important bioenergy traits for sweet sorghum. The sweet sorghum accessions had higher Cd concentrations in aboveground organs than grain sorghum accessions by no accident. On the other hand, the bioaccumulation factor (BCF), i.e. the ratio of Cd concentration in the whole aboveground of sorghum to soil Cd concentration, was significantly negatively correlated with the bioenergy traits, except for Brix. It was inferred that there would be a dilution effect on the capacity for Cd accumulation in sorghum.

Finally, as herbaceous annual grass, sweet sorghum can be completely removed together with the roots after harvest every year to achieve an efficient and thorough phytoremediation effect. Bioenergy crops including *Miscanthus*, *Pennisetum purpurem*, and *Arundo donax* have also been reported to have the capacity to absorb and fix HMs [78–80]. However, they are deep-rooted perennial grasses, and Cd is primarily accumulated in their underground parts. On the one hand, they may not be in full production and do not fully develop their rhizomes or the root system for phytoremediation in the first year of planting [79]. On the other hand, their large underground organs are difficult to completely remove after years of planting, hence the heavy metals-containing remainder in soil will pose a continuous threat to the environment. Additionally, phytoremediation of Cd-polluted soil by woody plants such as *Eucalyptus, Salix*, and *Populus* carries many year-consuming and requires a high cost [81–83].

3.5 Potential bioethanol yield of sweet sorghum under Cd stress

Sweet sorghums grown in Cd-contaminated soil are not suitable for the production of food or feed, but offer a promising bridge between phytoremediation and bioethanol production (as shown in Fig. 5a). Previously, the bioethanol yield of sweet sorghum under Cd stress was roughly estimated based on plant dry weight in pot test and the theoretical ethanol production per hectare. It was predicted that sweet sorghum treated with 1, 5, 10, 30, 50, and 100 mg/kg Cd polluted soil could produce ethanol of 3.65, 3.05, 3.14, 2.69, 1.15 and 0.41 t/ha, respectively (Fig. 5b) [65]. Furthermore, Liu et al. [21] chose to perform the theoretical calculation of ethanol yields from the cellulose, hemicelluloses, starch, and total soluble

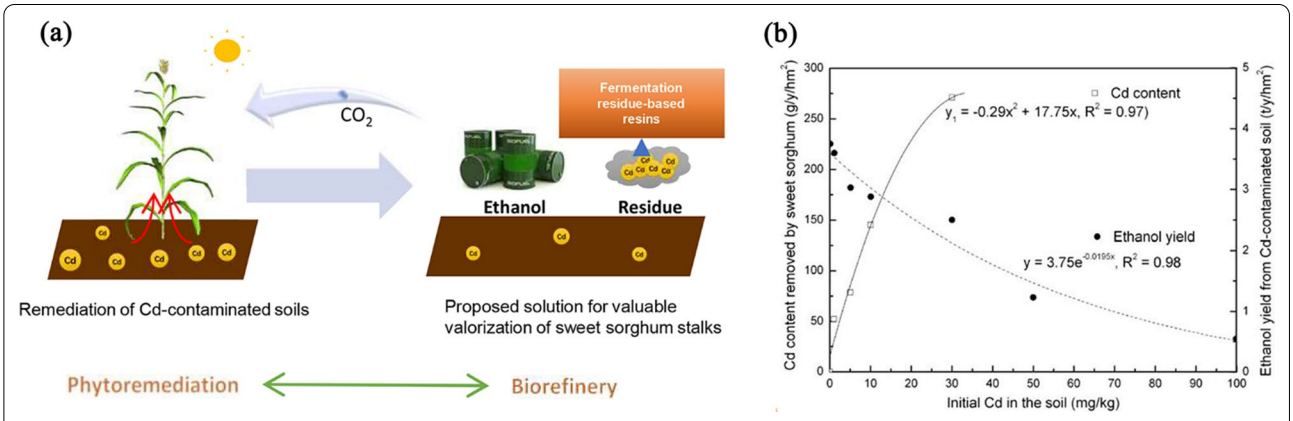

Fig. 5 Bioenergy potential of sweet sorghum for Cd-polluted soil phytoremediation. **a** Proposed strategy of using sweet sorghum integrated with phytoremediation and biorefinery. Reprinted from [22], Elsevier B. V., Copyright (2021). **b** Predicted removable Cd content and ethanol yield of sweet sorghum at different Cd concentration levels. Reprinted from [65], Taylor & Francis Ltd (http://www.tandfonline.com), Copyright (2015)

sugars contents in the five selected sorghum accessions. Assuming the sowing density of 165,000 plants/ha and double-cropping a year, sweet sorghum harvested from Cd-contaminated agricultural field (2.80 and 3.03 mg/kg Cd in soil) would produce 17.4–25.2 t/ha ethanol in total. Specifically, Xiao et al. [22] comprehensively investigated the biomass yields of sorghums and the components of stalks under large-scale field planting with soil Cd concentration of 0.25 and 0.96 mg/kg. The total theoretical bioethanol yields of sorghum stalks achieved 5510–7510 L/ha (4.36–5.93 t/ha) from one harvest. In addition, it has been reported that the stalks of sweet sorghum under Cd treatment (2.34–33.6 mg/kg) could be utilized by advanced solid state fermentation technology and presented no effect on sugar utilization rate as well as ethanol conversion rate during fermentation [71]. From the above, it is probable to pursue both environmental safety and energy benefits adopting phytoremediation sweet sorghum.

4 Bioethanol production from SSS

SSS is a good feedstock containing abundant soluble sugars and lignocellulosic biomass for 1G and 2G bioethanol production respectively (as demonstrated in Fig. 6). The production of sugar-based bioethanol can be directly achieved via microorganism fermentation, while the lignocelluloses require the pretreatment as well as the saccharification and hydrolysis strategies for 2G bioethanol production [24]. In this part, the bioethanol production from SSS will be discussed from three aspects: soluble sugars, sweet sorghum bagasse (SSB), and straw.

4.1 Soluble sugars to bioethanol

Most SSS contain approximately 15–40% soluble sugars on a dry mass basis, with some varieties containing up to 50% soluble sugars, primarily sucrose, glucose, and fructose [34, 85–92]. The total soluble sugar contents and the respective proportions of sucrose, fructose, and glucose in SSS are determined by genotype, planting year (environment), and phenological stage [49, 92–97]. In order to acquire the fermentable soluble sugars, the traditional and the most common approach is to mechanically press the stalks to release the saccharine juice. However, the crushing process is labor and energy intensive, and the juice recoveries of sweet sorghum from normal roller mills are generally below 60% [32, 98, 99]. Compared with sugarcane, the leaves left on stalks as well as the comparatively high contents of fiber and pith of sweet sorghum will limit the juice extraction yields and purities [100]. Additionally, the juice spoilage resulting from contaminating bacteria throughout storage and the juice clarification are also two significant issues [101]. For full utilization of the soluble sugars, other approaches have also been developed such as diffusion methods and solid state fermentation (SSF).

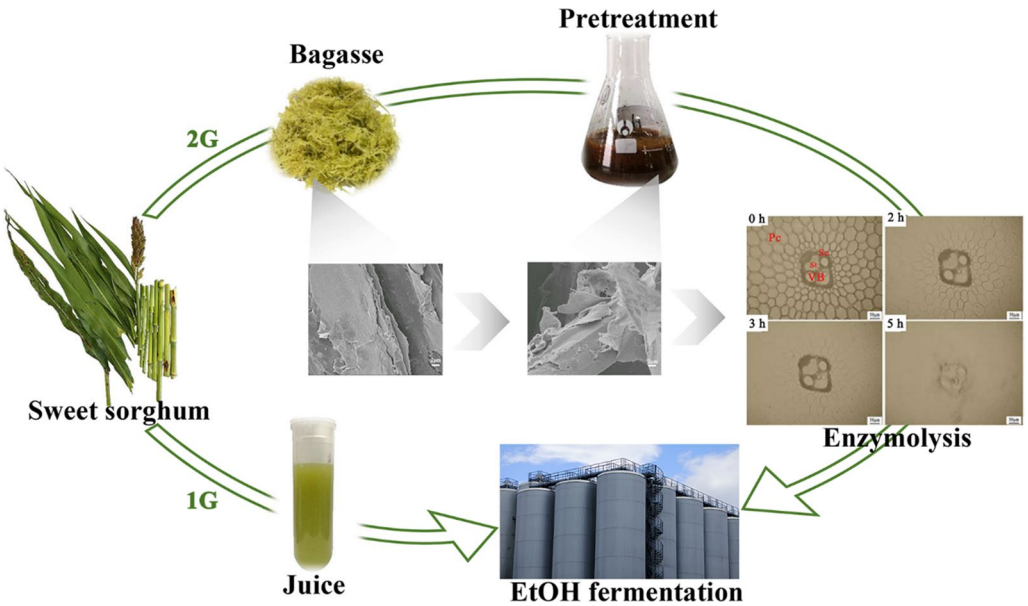

Fig. 6 Process flow of sweet sorghum conversion into bioethanol. The 'Enzymolysis' photograph is a real-time imaging of pretreated sweet sorghum straw (transverse section) during enzymatic hydrolysis. Adapted from [84], BioMed Central Ltd, Copyright (2019)

4.1.1 Liquid state fermentation

Contents of total soluble sugars in sweet sorghum juices are in the range of 110–190 g/L [98, 99, 102–106]. The fermentation of juices to ethanol has been extensively studied and established, and yeast (*Saccharomyces cerevisiae*) fermentation is the principal mechanism, that can efficiently convert sugars to ethanol under anaerobic conditions. As demonstrated in Table 2, yeast fermentation is capable to reach ethanol yields higher than 90% of the theoretical value, and the optimal fermentation temperature is around 30 °C, with the expected pH range of 4.0–5.2. The engineered microorganisms *Escherichia coli* could also be used for sweet sorghum juice fermentation, but with poor performance in sucrose utilization [105].

The laboratory-scale fermentation studies performed as liquid batch fermentation have evaluated the performance of sweet sorghum juices in ethanol fermentation, reaching up to the best fermentation efficiency of 94% [98, 99]. Fed-batch fermentation has been introduced to avoid the repressive effects of high product concentration and increase the conversion efficiency [102]. Continuous fermentation may minimize the concentration of inhibitory compounds, but the long cultivation times pose a high risk of outside contamination [100]. The repeated-batch fermentation is proposed as an extension, which drains the fermented juice at regular intervals and reuses the yeast cells recovered from the preceding fermentation broth for the next batch. This process offers many benefits including eliminating the costly re-sterilization steps and no requirement of inoculum preparation, leading to an enhancement in ethanol productivity. Besides, repeated-batch fermentation is able to use the sweet sorghum juice concentrated by the membrane separation system without any addition of exogenous nutrients [107–110]. To avoid the reduction in yeast cell concentration in repeated-batch process, the immobilized yeast cell systems are developed. Ethanol fermentations by immobilized yeast from stalk juice of sweet sorghum were effective, and the application of fluidized bed reactor significantly shortened the fermentation time [111, 112]. Considering the instability and high cost of conventional immobilization methods (cell entrapment on k-carrageenan or Ca-alginate), porous natural lignocellulosic materials such as corncob and SSS were employed as the carriers for cell immobilization, achieving high ethanol yields in sweet sorghum juice fermentation [113, 114]. Very high gravity (VHG) fermentation produces ethanol from mashes containing at least 250 g/L sugars with high productivity, therefore it has been described as "productive, water-saving, and cost-effective technology". Under appropriate aeration and nutrient supplementation in VHG conditions, the maximum ethanol concentration and yield in sweet sorghum juice fermentation could reach over 120 g/L and 99%. In addition, the high osmotic conditions will reduce the risk of bacterial contamination [115–117].

Diffuser extraction is a common technology in the sugar industry that typically achieves greater sugar extraction efficiency than juice extraction by crushing. In the cane sugar industry, diffusers can recover up to

Table 2 Summary of literatures on sweet sorghum juice fermentation

Fermentation mode	Microorganisms	Fermentation conditions	Time (h)	Initial total sugar (g/L)	Ethanol			References
					P (g/L)	Q_p (g/L/h)	Yield (%)	
Batch	Alcohol yeast Ethanol Red	pH 4.2, 30 °C, 150 rpm	72	200	–	–	93–94	[99]
	Baking yeast	pH 4.5, 30 °C, 100 rpm	24	110–191	43–82	–	68–94	[98]
	S. cerevisiae JP1	pH 4.5, 37 °C, 200 rpm	11	162	72	6.5	87	[104]
Fed-batch	*S. cerevisiae* TISTR 5048	pH 4.8, 30 °C, static	108	240	120	1.11	94	[102]
Repeated-batch	*S. cerevisiae* SSJKKU01	pH 4.0, 32 °C, 200 rpm	231 (8 cycles)	180–217	105	2.16	84	[107]
	S. cerevisiae BY4741	pH 5.2, 30 °C, 35 rpm	5*48	270	114	2.37	89	[108]
	S. cerevisiae BY4741	pH 5.2, 30 °C, 35 rpm	5*24	228	102–110	–	84–90	[109]
	S. cerevisiae F118	pH 5.2, 30 °C, 150 rpm	5*24	188	100	4.18	69–79	[110]
Immobilized yeast fermentation	*S. cerevisiae* CICC 1308	pH 5.0, 37 °C, 200 rpm	11	69	33	3.0	93	[111]
	S. cerevisiae Nanyang	pH 4.0, 32 °C, 150 rpm	5	111	49	–	92	[112]
Immobilized yeast in repeated-batch	*S. cerevisiae* TISTR 5048	pH 4.0, 30 °C, static	8*48	240	97	2.02	94	[113]
	S. cerevisiae NP01	pH 4.0, 30 °C, static	8*72	230	99	1.36	92	[114]
Very high gravity	*S. cerevisiae* NP01	pH 4.9, 30 °C, static	60	286	121	2.01	99	[115]
	S. cerevisiae NP01	No pH adjustment, 30 °C, 100 rpm	60	280	126	2.11	98	[116]

* P, ethanol concentration; Q_p, volumetric ethanol productivity

98% of the sugar while requiring simpler operation and maintenance, lower energy consumption, and lower costs than milling [100, 118]. In the diffusion process, raw materials are reduced to uniform geometric size and then passed through a series of gradient solutions that dissolved molecules [119]. The nonstructural carbohydrates in SSS can be easily extracted by water, and it has been reported that the water extraction recovered 2.5 times more sugar mass from SSS than press juice [89, 120]. The diffusion extraction method is applicable to both fresh SSS and dried ones, as well as to sorghum bagasse [121]. The extracted sugar solution can be fermented in liquid state as sweet sorghum juice, and would not impact the fermentation efficiency. Moreover, the liquid could even be incorporated into the dry-grind ethanol process or hemicellulosic sugar streams obtained through the steam treatment to enhance bioethanol yields [90, 120, 122]. A diffusion process is reported combining the utilization of starch in the panicles and soluble sugars in the stalks of sweet sorghum, realizing the high efficiencies for starch conversion (96%) and sugar recovery (98.5%) [119].

4.1.2 Solid state fermentation (SSF)

SSF has been defined as the bioprocess carried out in the absence, or near-absence of free water, involving the growth and metabolism of microorganisms on solid matrix [123]. Contrary to liquid state fermentation, the SSF of stalks directly converts the free sugars to ethanol, skipping the juice squeezing or sugar extraction. The SSF technology has continued to build up credibility in fuel ethanol production from sweet sorghum due to its higher sugar utilization and ethanol yield, lower energy expenditure and capital cost, and reduced water usage and wastewater output [124]. Previous studies explored the bioethanol production from fresh SSS or dry stalk particles by static SSF in laboratory scale, while investigating the influence of diverse process parameters such as particle size, yeast inoculation rate, temperature, and moisture content. And the maximum ethanol yields of 7.9 g-ethanol/100 g-fresh stalk and 0.25 g-ethanol/g-dry stalk were obtained [125–127]. Various thermotolerant yeasts are frequently used in SSF for sweet sorghum ethanol production, such as yeast AF37X [125], *Issatchenkia orientalis* IPE 100 [127], and S. cerevisiae TSH3 [128], while zygomycetes fungus *Mucor indicus* could also be an option [85].

Nevertheless, the absence of free water during SSF leads to poor heat removal, posing serious mass and heat transfer challenges for the industrial-scale operation of SSF. Other challenges including high viscosity, difficulty in fermentation control and solid handling, and limited types of microorganisms also impede large-scale production [41]. To achieve a cost-effectively system for commercial bioethanol production from SSS, advanced solid-state fermentation (ASSF) technology has been established and continuously improved. A rotary drum fermentation reactor was specially designed for efficient mass control and heat transfer; a *Saccharomyces cerevisiae* strain TSH-SC-1 with preeminent ethanol fermentative capacity and ability to withstand stressful SSF conditions was identified; the distillation kinetics in batch solid-state distillation to extract ethanol from fermented sweet sorghum bagasse was investigated [41, 124, 129, 130]. A commercial demonstration scale 550-m^3 rotary-drum fermentation system has already been constructed, fermenting up to 96 tons of crushed sweet sorghum within 20 h [124]. Besides, the ASSF technology could be combined with the alkaline pretreatment of sweet sorghum bagasse and C5-C6 co-fermentation in a whole process, and 91.9 kg ethanol/ton fresh SSS would be obtained under optimal conditions [131–133].

4.2 SSB to bioethanol

Sweet sorghum bagasse (biomass residue after juice extraction) is a promising feedstock for 2G bioethanol production, which primarily consists of cellulose, hemicelluloses, and lignin as illustrated in Fig. 7a. The raw SSB also contains some residual soluble sugar fraction (25–29%), and hot-water washing is an effective recovery method [121, 134, 135]. For the production of ethanol from SSB, cellulose and hemicelluloses must be disassembled into their corresponding pentose and hexose sugars before fermentation. However, the intricate structure of lignocellulosic biomass generates recalcitrance to chemicals or enzymes, resulting in critical challenge in the conversion processes of bioethanol [136]. The crucial factors affecting the biomass enzymatic digestibility include cellulose fiber crystallinity (CrI), sheathing and protection of both hemicelluloses and lignin, and porosity [137, 138]. Therefore, the SSB needs to be subjected to an effective pretreatment process to reduce the crystallinity, alter or remove hemicelluloses and lignin, and increase the accessible surface area to enzyme. The methods reported for the pretreatment of SSB can be categorized as physical (e.g. mechanical crushing, milling, irradiation, and sonication); chemical (e.g. acid, alkaline, peroxide, organic solvents, and ionic liquids); physico-chemical (e.g. hydrothermal treatment and steam explosion); biological; and other combined approaches. Besides, the pith and rind parts of sorghum stem are composed of different cell types, leading to the heterogeneity in chemical composition and biomass recalcitrance [139]. Furthermore, the cuticular waxes from sweet sorghum stem could inhibit the fermentation of acetone–butanol–ethanol to a certain extent [140]. To improve the utilization of sorghum stems, appropriate processing may be required to

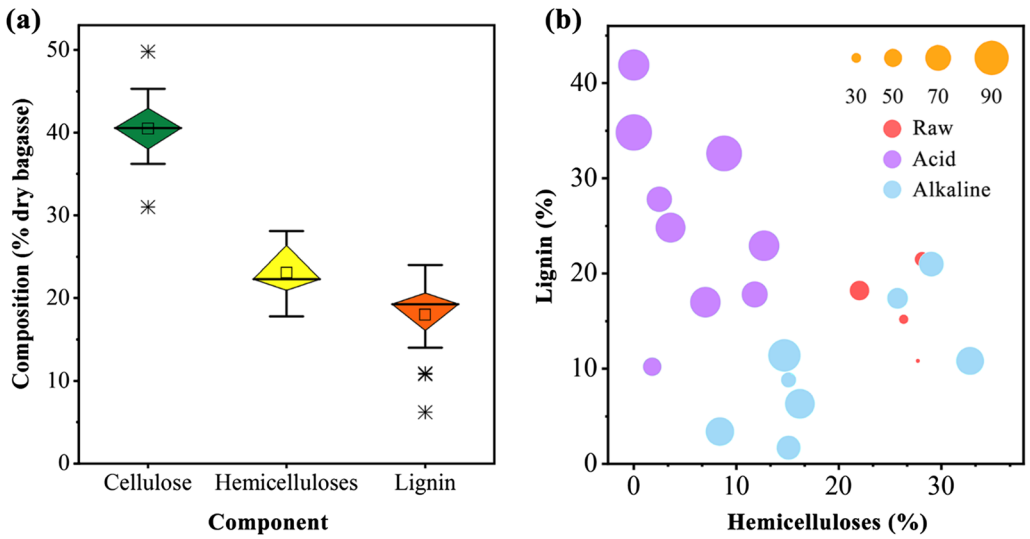

Fig. 7 Chemical composition and pretreatment effects of sweet sorghum bagasse. **a** Variations of components of dry bagasse [84, 93, 103–106, 121, 132, 141–152]. **b** Effects of acid/alkali pretreatments on the removals of hemicelluloses and lignin, and the ethanol yields of pretreated SSB [84, 104, 106, 132, 141, 143, 144, 146, 147, 149, 150, 152, 153]. The area of the circle represents the corresponding percentage to the theoretical ethanol yield. Partial ethanol yields were estimated based on the cellulose hydrolysis yields and enzymatic hydrolysis efficiencies

eliminate the negative effects caused by the rind region in bioethanol production.

4.2.1 Physical pretreatment

The physical or mechanical treatment is the first step for biorefinery processing. Methods such as chipping, milling, and grinding can be applied to effectively reduce the particle size of SSB, and also contribute to the reduction of cellulose crystallinity as well as the degree of polymerization (DP) [45, 138]. Particle size reduction increases the surface area and alleviates physical hindrances of raw biomass, thereby improves the subsequent pretreatment effect, enzyme accessibility, and the efficiency of enzymatic hydrolysis [26, 154]. Nevertheless, the comminution process of lignocelluloses is energy intensive, hence the processing needs to be considered with both biomass characteristics and the final particle size required [138]. Other forms of physical techniques such as ultrasonic [155], microwaves [135, 154], heavy ion beams irradiation [156], and gamma rays [157] have also been experimented for sweet sorghum pretreatment. However, there is no doubt that these methods will be costly to use on a large scale, along with the security risks.

4.2.2 Chemical pretreatment

Some chemicals are applied to pretreatment for efficient destruction of the native lignocellulosic structure and piercing the shields composed of lignin and hemicelluloses. The processes and pretreatment effects of recently reported chemical pretreatments of SSB are listed in Table 3. Indeed, SSB can be directly acid hydrolyzed into C5 and C6 sugars under relatively high acid concentration and long hydrolysis time treatments, but the sugars would also degrade into inhibitors under these harsh conditions and cause carbohydrates loss [158]. Therefore, the most established and common method for SSB producing bioethanol is pretreatment with dilute acids or alkalis under relatively mild conditions followed by enzymatic digestion.

Based on the previous research results, the effects of acid/alkaline pretreatments were visualized as Fig. 7b. While the mechanisms of the two pretreatment approaches are different, both are effective in improving the accessibility of cellulose and thus enzymatic efficiency. In acid pretreatment, H_2SO_4, HCl, CH_3COOH, and H_3PO_4 are generally exercised for hemicelluloses hydrolysis [143, 148–150]. Meanwhile, the xylan solubilization during acid pretreatment causes the collapse and porosity on the surface of the originally compact SSB fibers [84, 159]. On the other hand, alkaline (e.g. NaOH, $Ca(OH)_2$, and $NH_3{\bullet}H_2O$) pretreatment can cleave the ester bonds, weaken the hydrogen bond between hemicelluloses and cellulose, and lead partial lignin and hemicelluloses in the SSB removed, thereby getting rid of the lignin barriers and increasing the porosity of the biomass [84, 144, 146, 153].

Other chemical pretreatments such as H_2O_2, ionic liquids [BMIM] Cl, glycerol, 1-butanol were also feasible for sorghum bagasse, but their process costs are expensive [84, 141, 153, 160]. Simulated green liquor pretreatment

Table 3 Chemical pretreatment and bioethanol fermentation of SSB

Pretreatment	Chemical compositions %			Enzymes/Fermenting microorgansisms	Results	References
	Cellulose	Hemice-llulose	Lignin			
Washed baggasse	45.3	26.3	15.2	Cellulase	Cellulose conversion of 27%	[141]
Crude baggasse	37.7	28.1	21.5	Cellulase (*T. longibrachiatum* LC-M4)	Enzymatic hydrolysis efficiency of 43%	[84]
Mixed with H_3PO_4 (85%) at 50 °C for 30 min and washed with cold acetone	52.2	13.1	24.2	Celluclase (Celluclast 1.5L) and β-glucosidase (Novozyme 188)/*Mucor hiemalis* CCUG 16148	Enzymatic hydrolysis yield of 79%; 76% of the theoretical ethanol yield	[143]
0.5% H_2SO_4, heated up to 180 °C and held for 5 min, then cooled to room temperature at 10 °C/min	65.8	0	34.8	SSF: cellulase (NS50013), gluosidase (NS50010), and hemicellulose (NS22002)/*Saccharomyces cerevisiae* (ATCC 24858)	The ethanol yield, concentration, and production rate were 89.4%, 38 g/L, and 1.28 g/L/h, respectively	[149]
5% (w/w) CH_3COOH, heated up to 180 °C and held for 5 min	53.1	8.8	32.6	Fed-batch SSF: cellulase (NS50013), gluosidase (NS50010), and hemicellulase(NS22002)/*Saccharomyces cerevisiae* (ATCC 24858)	Ethanol yield of 89%	[150]
1% $Ca(OH)_2$, at 25 °C for 24 h	48.2	25.7	17.4	Cellulase (CTec 3)	Cellulose conversion of 61%	[146]
2% NaOH (w/v), at 100 °C for 1 h	71.4	16.2	6.3	Cellulase (*T. longibrachiatum* LC-M4)	Enzymatic hydrolysis efficiency of 86%	[84]
15% aqueous ammonia solution, heated at 120 °C for 60 min	48	29	21	Cellic CTec2	Cellulose and xylan hydrolysis efficiency of 72% and 62%; total sugar yield of 356 mg /g biomass	[153]
Ionic liquids [BMIM] Cl pretreatment in a 110 °C oil bath at 120 rpm for 1 h	48.8	16.7	25.3	Cellulase	Cellulose conversion of 41%	[141]
Simulated green liquor (Na_2CO_3 and Na_2S), at 160 °C for 110 min	–			Cellic CTec2	Total sugar yield of 83%	[134]
10% (v/v) H_2O_2, at 100 °C for 1 h	54.6	24.5	11.6	Cellulase (*T. longibrachiatum* LC-M4)	Enzymatic hydrolysis efficiency of 67%	[84]
60% (w/w) glycerol, heated at 190 °C for 60 min	36	19	21	Cellic CTec2	Cellulose and xylan hydrolysis efficiency of 78% and 46%; total sugar yield of 313 mg /g biomass	[153]

(Na_2CO_3 and Na_2S) on SSB could dissolve lignin while preserving carbohydrates. As a result, the predicted total sugar yield could reach 83.2% at optimum condition (160 °C for 110 min, liquid/solid ratio of 7, total titratable alkali of 18%, and sulfidity of 40%) [134]. Still, chemical pretreatments have some disadvantages, such as the equipment requirement, carbohydrate loss, generation of toxic chemicals, and relative high cost.

4.2.3 Physico-chemical pretreatment

Physical–chemical pretreatment of SSB is mainly achieved by liquid hot water (LHW) pretreatment, steam explosion, and ammonia fiber explosion (AFEX). Comparing with chemical methods, the LHW pretreatment with no chemical addition and little erosion on equipment is becoming attractive. During the LHW pretreatment, the hemicelluloses can be well solubilized with the majority of pentosan recovered, while avoiding the generation of fermentation inhibitors. Simultaneously, liberation of acids during hemicelluloses hydrolysis and the minor loss of cellulose would enhance the following enzymatic hydrolysis [121, 161, 162]. After pretreatment with LHW at a step-change flow rate (184 °C for 8 min at 20 mL/min, then 10 min at 10 mL/min) and 72 h enzymatic digestion, the SSB could produce 83.7% of the total sugars [161]. Steam treatments of SSB can be performed with or without catalyst, which heat biomass by saturated steam and then decompress the pressured system to achieve an explosion effect. This process allows a better fractionation of SSB and solubilization of hemicellulose and even lignin [89, 105, 138, 163]. Zhang et al. [141] revealed that the steam-exploded SSB attained the maximum cellulose conversion of 70%, which was about 1.6 times higher than that of the untreated sample (27%). Li et al. [164] optimized the AFEX pretreatment for SSB (120% moisture content, 2:1 ammonia to biomass loading, 140 °C, and 30 min residence time), and achieved the glucan and xylan conversion about 80% and 90%, respectively.

4.2.4 Biological pretreatment

As the most similar to the natural conversion route of lignocellulosic biomass, biological pretreatment commonly represents eco-friendly. In biological pretreatment, fungi are the most suitable and efficient candidates, which produce enzymes that can degrade hemicelluloses, lignin, and polyphenols efficiently. Besides fungi, some microbial consortium, bacterial systems, and crude enzymes such as lignin peroxidases, Mn peroxidase, and laccases are also applied to destruct the lignocellulosic biomass. Whereas, the biological approach is generally slower and has lesser efficiency than other pretreatments for industrial purposes [45, 138]. Latterly, Mishra et al. [165] found that fungus *Coriolus versicolor* could pretreat the SSB selectively due to its high ligninolytic and low cellulolytic enzyme production. In addition, the maximum lignin degradation was achieved with syringic acid supplement, resulting in a 1.9 times higher sugar yield than untreated SSB.

4.2.5 Combined approaches

The mixture of one or more pretreatment methods are also applied for SSB pretreatment, such as physical-biological, chemical-physical, chemical-biological, and thermal-chemical pretreatments [45, 154, 166, 167]. Besides, there are also studies using multi-step chemical methods for pretreatment of SSB [144, 147, 152, 168]. Koo et al. reported a modified two-stage autohydrolysis combined with mechanical treatment, achieving the total sugar recovery of 83.9% to the total available sugars in SSB [121]. The selection of the pretreatment method should aim at minimizing additional energy consumption and having good compatibility with the next operation [169]. Nevertheless, the implementation of several, dissimilar pretreatment methods usually introduces additional requirements and costs, which is not desirable. Comprehensive consideration of pretreatment effect and cost is more conducive to industrial promotion and application.

4.3 Stalk to bioethanol

Traditional pretreatments such as acid and alkaline processes would decrease bioethanol yields of SSS since the degradation of free sugars. Recently, new approaches are developed to pretreat SSS in one step, thus avoiding the necessity of juice extraction. Nozari et al. [88] proposed an improved organosolv pretreatment for the bioconversion of SSS into bioethanol and biogas. The maximum gasoline equivalent (0.249 L/kg) was obtained when using the mixture of EtOH and isopropanol (IPOH) (60:20) in the presence of 1% H_2SO_4 treated SSS at 140 °C for 30 min. Damay et al. [170] put forward a novel approach based on steam pretreatment to recover the free and hemicellulosic monomeric carbohydrates from fresh sweet sorghum in one stage. Under the optimal operating conditions (180 °C for 3 min), 30% monomeric carbohydrates were recovered based on the dry weight of sorghum with the lowest composition of inhibitors. And the recovered carbohydrate streams achieved a maximum ethanol yield of above 95%. Williams et al. [87] have firstly investigated the integration of soluble sugar extraction and mild NaOH pretreatment using counter-current solid–liquid extraction technology, and developed a novel processing scheme utilizing both extractable and structural carbohydrates to produce biofuels. The integrated deconstruction and extraction were conducted under alkaline conditions, employing the pretreatment

with the equivalent of 0.06 g NaOH/g biomass at 80 °C as one of the stages during counter-current extraction. The high pH (> 12) liquor from the pretreatment stage was progressively neutralized over the subsequent extraction stages, finally dropping to an appropriate pH of 5.5. The mixed sugar solution of the extraction liquor and cellulosic hydrolysate was found to be fermentable without detoxification. A high bioethanol titer of 80 g/L could be achieved by fermenting concentrated sugar stream.

5 Screening and breeding of ideotypes

World collection of sorghum consists of 235,711 accessions, exhibiting huge genetic diversity and resources towards the variations in climatic conditions of different regions [31, 171]. Conventional breeding techniques such as hybridization-based methods are successful in improving sorghum varieties [172]. With recent developments of sorghum research in the field of molecular biology, including the survey of mutant populations, dissection of quantitative trait loci (QTLs), identification, and isolation of genes controlling important agronomic traits, the process of molecular breeding is promoted [31]. DNA marker technologies and genetic transformation techniques are now increasingly employing for sorghum improvement to supplement traditional breeding methods [173, 174]. Previously, a suite of biofuel-related traits and their genetic determinants in sweet sorghum were identified, such as sugar content in stems, plant height, flowering time (maturity), plant architecture (leaves, root, and stem), and biomass bioconversion efficiency. Targeted genetic modulation can operate on these traits and pose a potential pathway to optimize sweet sorghum for biofuel production [175, 176].

For the optimum results of phytoremediation and bioethanol production, the screening and breeding of sweet sorghum ideotypes is a cornerstone. This targeted breeding aim requires for high Cd uptake, high biomass, high carbohydrates yield, and good adaptability to diverse agroclimatic conditions. As discussed in the above section on characteristics of sweet sorghum in Cd phytoremediation, the total Cd removal is mainly achieved by stems due to their high yields. Therefore, cultivars with high Cd translocation factor and stalk yields are more suitable for Cd removal. Besides, considering the strong correlation between bioenergy-related agronomic traits and aboveground Cd accumulation of sorghum [21], the screening of traits such as biomass, internode numbers, stem Brix, and plant height will be of substantial assistance. Feng et al. [67] reported that many DEGs relating to differential Cd accumulation in sweet sorghum were found to be linked with cell wall modification, including genes involved in cell wall biogenesis and modification as well as cell wall macromolecule (pectin, cellulose, lignin, and suberin) catabolic process. Additionally, partial MicroRNAs and their target genes of sweet sorghum that might function in Cd accumulation have been revealed [68]. These findings provide useful references for improving phytoremediation ability of sweet sorghum through genetic engineering.

A previous study showed that the SSB had a relatively higher biomass enzymatic digestibility than *Miscanthus* and wheat species. It also demonstrated that the arabinose substitution degree of the non-KOH-extractable hemicelluloses in sweet sorghum exhibited a negative correlation with the raw material CrI, while also positively affected biomass enzymatic digestibility [91]. These results are highly probable to be related to the cell wall structure of sorghum. A unique model of sorghum cell wall architecture has been proposed that xylan in sorghum secondary cell walls is mainly in a three-fold screw conformation due to dense arabinosyl substitutions, with close interacting with amorphous cellulose but rarely docking on the hydrophilic surface of crystalline cellulose. Besides, sorghum secondary cell walls have a larger proportion of amorphous cellulose relative to dicots. Compared with the xylan-cellulose interactions in dicot plants and softwoods which are dominated by hydrogen bonds between two-fold screw xylan and cellulose fibrils on the hydrophilic surface, those in sorghum secondary cell walls dominated by interactions between the amorphous cellulose and three-fold screw xylan are significantly weaker [177]. These discoveries could offer fundamental guidance for genetic modification of plant cell walls oriented to reduce biomass recalcitrance and improve the bioenergy conversion efficiency of sweet sorghum.

6 Conclusions and perspectives

Sweet sorghum is a resilient and fast growing C_4 plant, with a wide adaptability to different environmental conditions and relatively lower agronomic requirements. It can produce high biomass with abundant soluble sugars in the stalk, making a promising feedstock for bioethanol production. Although sweet sorghum is not hyperaccumulator, it can grow normally and produce adequate biomass under moderate Cd pollution. After maturity, most of the absorbed Cd is maintained in the aerial parts especially stems that can be removed entirely for bioethanol production, thus entering the energy chain rather than the food chain. Therefore, phytoremediation of Cd-polluted arable lands by sweet sorghum is a cost-effective and ecofriendly pathway. Despite the achievements already made, some essential issues still exist and demand for emphasis.

In terms of the phytoremediation with sweet sorghum, the existing pot tests and field trials show that

different sweet sorghum cultivars exhibit huge diversities in Cd tolerance and biofuel-related traits. Therefore, screening and selection of appropriate sweet sorghum varieties with high Cd absorption capability, high bioethanol yield, and superior adaptability to diverse agroclimatic conditions are extremely significant for practical application. Besides, the mechanism of Cd tolerance in sorghum remain not completely clarified, which requires more multidimensional and in-depth studies to figure out.

As for the further utilization of SSS after phytoremediation, the technology for the complete processing of bioethanol production is not well developed. Most published studies were conducted on a laboratory scale. Further research should strengthen the comprehensive use of sweet sorghum, integrate the 1G and 2G bioethanol production, and increase sharing of existing critical factory facilities, with the goal of minimizing investment and enhancing economic feasibility. It is extremely important that Cd is one of the most mobile HMs in the environment. Since there is still a serious gap of safe biorefining of Cd-containing raw materials left to be filled, research on the migration pattern of Cd and the ultimate treatment should be expanded, ensuring no secondary pollution.

Abbreviations

Cd: Cadmium; SSS: Sweet sorghum stalk; 1G: First generation; 2G: Second generation; Chl: Chlorophyll; EdU: Ethynyl deoxyuridine; ROS: Reactive oxygen species; DEGs: Differentially expressed genes; KEGG: Kyoto Encyclopedia of Genes and Genomes; ACdA: Aboveground Cd accumulation; BCF: Bioaccumulation factor; SSB: Sweet sorghum bagasse; SSF: Solid state fermentation; ASSF: Advanced solid-state fermentation; LHW: Liquid hot water; AFEX: Ammonia fiber explosion; QTL: Quantitative trait loci.

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Authors' contributions

XMZ summarized the literatures and wrote the initial draft; SQ and HS optimized the figures and tables, and revised the draft; CWJ and PB revised the draft, mainly focusing on the language and logicality. DZY, YWB, and SZ critically reviewed and commented the manuscript in the prepublication stage. YTQ designed the outline of the draft, supervised and coordinated the execution of this research. All authors read and approved the final manuscript.

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Consent for publication

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Competing interests

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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