Current Research in Microbial Sciences 2 (2021) 100018

Contents lists available at ScienceDirect

Current Research in Microbial Sciences

journal homepage: www.elsevier.com/locate/crmicr

Bioprospecting of cowdung microflora for sustainable agricultural, biotechnological and environmental applications

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ARTICLE INFO

Keywords:
Biocontrol
Biosorption
Biodegradation
Biogas
Bioprocess
Bioremediation
Cow dung
Panchagavya

ABSTRACT

The review aims at highlighting the manifold applications of cow dung (CD) and CD microflora covering agricultural, biotechnological and environmental applications. The update research on CD microflora and CD in agricultural domain such as biocontrol, growth promotion, organic fertilizer, sulfur oxidation, phosphorus solubilization, zinc mobilization and underlying mechanisms involved in these processes are discussed. The significance of CD applications in tropical agriculture in context to climate change is briefly emphasized. The advances on genomics and proteomics of CD microflora for enhanced yield of enzymes, organic acids, alternative fuels (biomethane and biohydrogen) and other biocommodities, and environmental applications in context to biosorption of heavy metals, biodegradation of xenobiotics, etc. have been given critical attention.

1. Introduction

Cow dung (CD) or cow manure is the waste product of bovine animal species that include domestic cattle (cows, bullock, and buffalo), yak, and water buffalo. CD is the undigested residue of plant matter which has passed through the animal's gut and includes water (80%), undigested residues (14.4%), and microorganisms (5.6%). The pH of the CD varies from 7.1- 7.4 (Radha and Rao, 2014). The fecal matter in CD is rich in crude fiber (indigestible cellulose, hemicelluloses, pentosans, lignin), crude protein, and 24 types of minerals including nitrogen (N), phosphorus (P), potassium (K), iron (Fe), sulfur (S), magnesium (Mg), calcium (Ca), cobalt (Co), manganese (Mn), chlorine (Cl) (Garg and Mudgal, 2007; Randhawa and Kullar, 2011) and sloughed off intestinal epithelium. The portion of fecal matter derived from the rumen of cattle improves the constituents of CD by enriching with bile pigments (biliverdin), intestinal bacteria, and mucus.

CD is traditionally used as organic fertilizer in Asian and African agriculture for ages (Sawatdeenarunat et al., 2016). In addition to the nutritional contributions to the soil, CD enhances resistance in plants against pests and diseases, stimulates plant growth, along with P and S solubilization (Sharma and Singh, 2015). CD also harbors diverse groups of microorganisms that further enhance soil biogeochemical processes (Akinde and Obire, 2008). In Ayurveda (it is a system of traditional medicine that has historical roots in the Indian Subcontinent), different processed products obtained from cattle such as milk, curd, ghee, urine and by-product (dung) are widely used in medicinal formulations (Sharma and Singh, 2015).

The current status of CD as a bioresource for sustainable development has been briefly reviewed by Gupta et al. (2016). In this review, the various applications of CD and CD-based microorganisms' uses in agriculture, aquaculture, and bioprocesses have been outlined. Mandavgane and Kulkarni (2020) reviewed the valorization of cow urine and CD in the model biorefinery. However, several critical aspects such as microbial diversity, biodynamics preparation and uses of CD in agriculture, underlying mechanisms in bioprocesses, and biotechnological applications (i.e., enzymes, biomethane, and biohydrogen) and environmental applications are not fully discussed in these reviews. In this context, the present review provides a comprehensive discussion on the underlying mechanisms of CD microorganisms in agricultural, biotechnological, and environmental applications in a sustainable circular economy context.

Abbreviation: AD, anaerobic digesters; AP, apple pomace; ARB, antibiotic-resistant bacteria; ARGs, antibiotic-resistant genes; BOD, biochemical oxygen demand; C/N, carbon nitrogen ratio; CD, cow dung; CEC, cation exchange capacity; CDP, cow dung powder; DO, dissolved oxygen; EC, electric conductivity; IAA, indole-3-acetic acids; NPK, nitrogen, phosphorus, and potassium; NPP, net primary productivity; OM, organic matter; PGPR, plant growth promoting rhizobateria; PSM, P-solubilizing microorganisms; SGR, specific growth rate; SmF, sub-merged fermentation; SSF, solid sate fermentation; TOC, total organic carbon; TPPB, two phase partitioning bioreactor; TS, total solids.

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https://doi.org/10.1016/j.crmicr.2020.100018
Received 13 November 2020; Received in revised form 8 December 2020; Accepted 10 December 2020

2. Historical significance of CD

Ayurveda is one of the life sciences of the Vedic [The **Vedic** (c. 1500 – c. 500 BCE) was the period in Indian history during which the **Vedas**, the oldest scriptures of Hinduism, were composed] period (Patwardhan et al., 2005). *Panchgavya*, a term used in Ayurveda, describes the blend of five products/byproducts from cow [urine, milk, curd, clarified butter (ghee), and dung] (Garg and Mudgal, 2007; Sharma and Singh, 2015). *Panchgavya therapy* (cow-therapy) is widely practiced in India as an alternative therapeutic approach for sound human and livestock health, It's antimicrobial and antifungal properties have drawn attention among medical and veterinary professionals (Joseph and Sankarganesh, 2011).

3. Microbial diversity of CD

The microbial diversity of CD (coprophilous organisms) has received the attention of biologists since the last century (McGranaghan et al., 1999; Kim and Wells, 2016). The presence of naturally occurring beneficial microorganisms, predominately bacteria (bacilli, lactobacilli, and cocci), and some actinomycetes, fungi, and yeast have been reported in CD (Radha and Rao 2014; Sharma and Singh, 2015). CD harbors a rich microbial diversity containing almost 60 species of bacteria (i.e. *Bacillus* sp., *Lactobacillus* spp., *Corynebacterium* spp.), fungi (i.e. *Aspergillus, Trichoderma*), 100 species of protozoa and yeasts (i.e. *Saccharomyces* and *Candida*) (Gupta et al., 2016; Bhatt and Maheswari, 2019).

3.1. Bacteria

Although bacteria and fungi are both important contributors to the composting process of CD, bacteria are more abundant (Holman et al., 2016). The general microflora inhabitant of the cattle gut involves *Bacillus, Bifidobacterium,* and *Lactobacillus* (Teo and Teoh, 2013). Velazquez et al. (2004) identified a novel species of xylanolytic, facultatively anaerobic, motile, gram-variable, sporulated rod bacterium *Paenibacillus flaviporus* from fresh and aged CD based on 16S rRNA gene sequence analysis. Adegunloye et al. (2007) investigated microbial analysis of compost using CD as a booster. The compost supported a high population of bacteria mainly *Bacillus pumilus, Bacillus sphearicus, Bacillus macereans, Bacillus lateosporus, Micrococcus varians, Proteus mirabilis*, and *Enterobacter aerogenes*. Several bacterial species have been reported from CD such as *Citrobacter koseri, E. aerogenes, Escherichia coli, Klebsilla oxytoca, Klebsilla pneumonia, Kluyvera* sp., *Morgarella morganii, Pasteurella* spp., *Providencia alcaligenes, Providencia stuartii* and *Pseudomonas* spp (Sawant et al., 2007). The aerobic heterotrophic bacteria isolated were *Acinetobacter* spp., *Bacillus* sp., *Serratia* sp., *Alcaligenes* sp., and *Pseudomonas* sp (Akinde and Obire, 2008). In a later study from India, *Bacillus safensis* (PG1), *Bacillus cereus* (PG2, PG4 PG5), *Bacillus subtilis*(BD2) *Lysinibacillus xylanilyticus* (BD3), and *Bacillus licheniformis* (CPP1) were isolated and identified from CD (Radha and Rao, 2014). The pyrosequencing of 16S rRNA gene of bacteria obtained from bio-stabilization of CD during vermicomposting was analyzed and *Proteobacteria* were in the highest proportions (Lv et al., 2015).

3.2. Actinomycetes

Actinomycetes are members of a heterogenous group of Gram-positive, anaerobic bacteria accounted for a filamentous and branching growth pattern (Berkowitz, 1994). These actinomycetes are an integral part of CD those have been implicated in the production of unpleasant flavors, odors, and colors. Of the specific types of actinomycetes, *Nocardia* spp. are predominately present among CD microflora (Radha and Rao, 2014). Moreover, a very high number of nocardioform, *Rhadococcus coprophillus* have been isolated from the dung of domesticated herbivores (Rowbotham and Cross, 1977). Godden et al. (1983) reported nine species of actinomycetes in cattle manure; out of these *Micromonospora chalcae* and *Pseudonocardia thermophila* were cellulose decomposers. In a recent study, Semwal et al. (2018) isolated 30 actinomycetes species from fresh CD and all of them belong to *Streptomyces* spp. based on morphological and chemotaxonomic analysis (16S rDNA sequence).

3.3. Fungi and yeasts

Various authors reported different fungi from CD. For example, *Aspergillus niger, Aspergillus flavus, Aspergillus rapens, Aspergillus fumigatus, Rhizopus stolonifer, Mucor mucedo, Fusarium* spp. and *Vericosporium* spp. were reported in CD (Adegunloye et al., 2007); saprophytic fungi (yeast and molds) such as *Alternaria* sp., *Aspergillus* sp., *Cephalosporium* sp., *Cladosporium* sp., *Geotrichum* sp., *Monilia* sp., *Mucor* sp., *Penicillium* sp., *Rhizopus* sp., *Sporotrichum* sp., *Thamnidum* sp., *Candida* sp.,. *Rhodotorula* sp., *Saccharomyces, Sporobolomyces, Trichosporon,* and *Torulopsis* sp. were reported by others (Obire et al., al.,2008; Okwute and Ijah, 2014). Some fungi such as *Blastomyces* sp., *Botryodiplodia theobromae, Fusarium* sp., *Nigrospora* sp., *Penicillum chrysogenum, Penicillum glabrum, Pleurofragmium* sp., and *Trichoderma harzianum* isolated from CD were reported as petroleum oil-degraders in aquatic environments in Nigeria(Orji et al., 2012).

3.4. Genomics of CD microflora

Several factors determine the microbial community of CD. Diet is the major factor altering fecal microbial communities, while breed, age, gender and ecological factors are minor factors that influence fecal microbial communities (Kim et al., 2014). In most cases, fecal bacteria in cattle have been analyzed using culture-dependent methods that gave approximately 1% of the actual bacteria present in the animal gut (Dowd et al., 2008; Wiegel et al., 2008; Vaishnav and Demain, 2009; Callaway et al., 2010; Jami and Mizrahi, 2012). A bacterium with a similarity of *Clostridium cellulosi* was detected in the fermented CD by 16S rDNA analysis (Yokoyama et al., 2007

Dietary components of cattle influence the gastrointestinal microbial ecology and diversity in CD (Callaway et al., 2010; Kim and Wells, 2016). Kim et al. (2014) investigated bacterial diversity in CD fed with different diets (corn-based, forage diet) using metagenomics. Individual fecal samples from 333 cattle were analyzed. for determining the bacterial 16S rRNA gene amplicons. A total of 2,149,008 gene sequences were analyzed and two dominated phyla, i.e. *Firmicutes* and *Bacteroidetes* were found in all fecal samples. Girija et al. (2013) studied a detailed analysis of CD microbiota based on a culture-independent 16S rDNA approach. Total community of DNA was extracted from fresh CD and bacterial 16S rRNA genes were amplified, cloned and sequenced. This study detected *Acinetobacter, Bacillus, Stenotrophomona* and *Pseudomonas* that were producers of indole acetic acid (IAA) and siderophore (Kitamura et al., 2016). Pooja et al. (2015) constructed a metagenomic library by cloning CD metagenomic DNA fragments into pGX-1 vector containing green fluorescent protein (GFP). The clones expressing GPF from the library were screened on maltose induced fluorescence-activated cell sorter. One positive clone was isolated and the presence of 2031 bp open reading frame (ORF) designed as *amy* 1, encoded for periplasomic α-amylase. Many *Acinetobacter* and *Pseudomonas* isolated from CD have been reported to possess N_2- fixing and P solubilizing activity. Several genera of bacteria such as *Bacillus* and *Pseudomonas* were identified in this study known for antagonistic properties against bacteria and fungi (Lima-Junior et al., 2016). More recently, the microbial community structure of CD is analyzed through terminal restriction fragment length polymorphism (Bharti et al., 2016).

Recent technological advances in metagenomics have brought the field closer to the goal of restoring all genomes within microbial diversity of CD microflora at a much lower cost (Ercolini, 2013). However, there are some new informatics challenges (i.e., high throughput sequencing of amplified markers/DNA barcodes) that must be addressed to improve the understating of the complexity of CD microflora

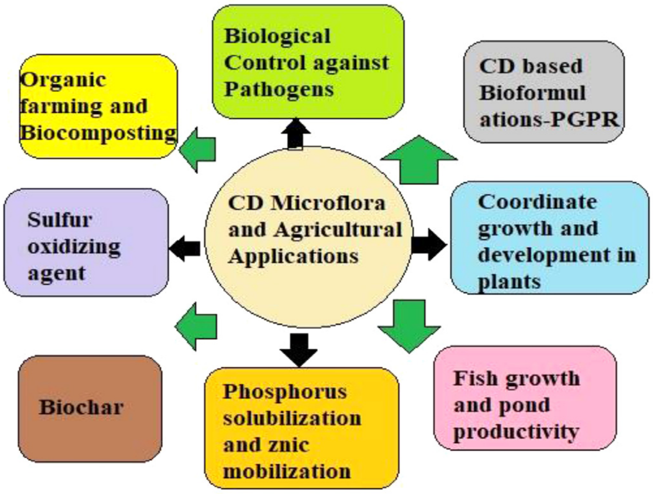

Fig. 1. Agricultural applications of cow dung microflora.

using metagenomics such as next-generation sequencing approaches (Scholz et al., 2012; Garza and Dutilh, 2015).

4. Agricultural applications

The CD microflora-based formulations (Fig. 1) having potential applications in agriculture, horticulture, and aquaculture are given in Table 1.

4.1. Biological control

Some of the bacteria isolated from CD have shown antagonistic effects against pathogenic fungi (Basak and Lee, 2001, 2002; Swain et al., 2008; Swain and Ray, 2009a, 2009b). One of the underlying mechanisms is that these antagonistic bacteria play a significant role by rapidly colonizing the surface area of the CD-treated seeds, thereby inhibiting the growth of pathogenic fungi (Swain and Ray, 2009a). Fusarium wilt is a serious problem that causes 30–100% crop loss (Sundaramoorthy et al., 2012). Cow urine and CD are capable of suppressing conidial germination and mycelia growth of *Fusarium oxysporum* f. sp. *cucumerinum* (Owen) that cause Fusarium wilt of cucumber (Basak and Lee, 2001; Basak et al., 2002). The *B. subtilis* strains CM1 and CM 3 isolated from CD inhibited the *in vitro* growth of fungi, *F. oxysporum* (25–34%) and *B. theobromae* (100%), postharvest rot pathogens of yam (*Dioscorea rotundata* L.) tubers (Swain et al., 2008; Swain and Ray, 2009a). Lytic enzymes such as chitinase, presumably along with antimicrobial metabolites, were involved in the inhibition of the growth of these fungi (Fig. 2) (Swain et al., 2008). Akhter et al. (2006) reported the inhibitory effect of CD on conidial germination of *Bipolaris sorokinjana* that causes common root rot of small cereal grains. In a recent study, out of 30 *Streptomyces* strains isolated from fresh CD, 15 strains (50%) showed antifungal activity (50–62% inhibition) against five fungal phytopathogens including *A. niger, Fusarium solani, F. oxysporum, Macrophomina phaseolina* and *Rhizoctonia solani* (Semwal et al., 2018).

The CD was reported to be effective for the control of bacterial sheath blight of rice caused by *R. solani* (Srivastava et al., 2010). The aqueous extracts of CD (0.5–5, w/v) found to be effective on four fungal species like *Alternaria alternata, F. oxyporium, Colletotrichum capsici* and *Curvularia lunata* for their germination attributes (Shrivastava et al., 2014). Ahuja et al. (2012) reported that CD showed a positive response in suppression of mycelial growth of plant pathogens, *F. solani, F. oxyporum* and *Sclerotinia sclerotiorum.* In a two year field experiment (2013 and 2014) conducted in China. *Streptomyces cochorusii* strain NF0919 and *Bacillus amyloliquefaciens* strain SB177 isolated from CD was found very effective in controlling rice sheath blight pathogen, *R. solani*. The field biocontrol efficacy after spraying 7 days in 2013 and 2014 was 78.4 and 98.1% with a crude extract from NF0919 culture filtrate and 71.1 and 94.2% with fresh cells of *B. amyloliquefaciens* strain SB177, provided better disease control than other fungicides (Jinggangmycin and/or Kresoxim-methyl, commercial antifungal agent widely used in China) (Yang et al., 2017).

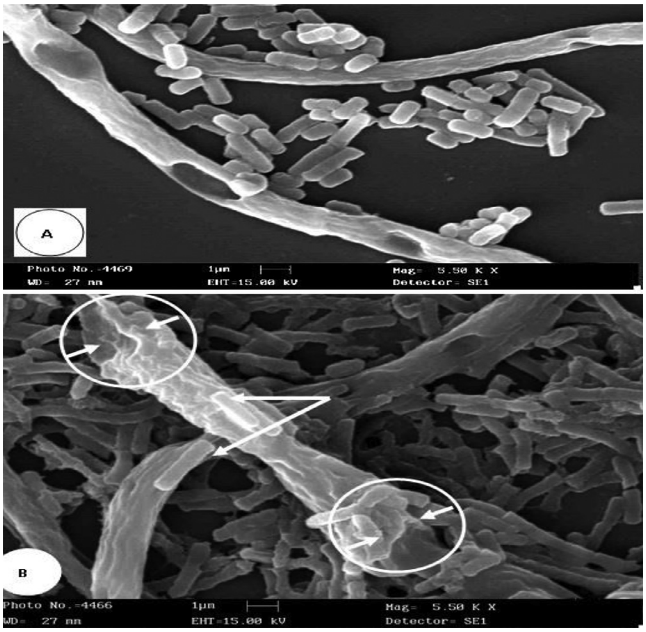

Fig. 2. Scanning electron micrograph (SEM) of *Fusarium oxysporium* sample collected at 12 h (A) and 36 h (B) after interaction with *Bacillus subtilis* CM1. The solid and dotted arrow shows the bacterial attachment with fungal hyphae and lytic mark hyphae. Circles indicate the complete lysis of fungal mycelium after 36 h of interaction.
(Source: Swain et al., 2008).

Nautiyal et al. (2013) predicted the probable mechanism of CD-mediated reduction of wilt in chickpea (*Cicer arietinum*). It was indicated that CD coating on chickpea seeds reduces activities of cell wall-degrading enzymes (hydrolases) in a transcriptional regulated manner, which in turn function as biocontrol measured for fungal growth in *C. arietinum* roots. Patel et al. (2016) reported for efficacy of CD and urine for controlling red rot diseases of sugarcane caused by *Colletrotrichum falcatum*. CD isolated strains, *Streptomyces cochorusii* NF0919 and *B. amyloliquefaciens* SB177 were found as potential bocontrol agent against the rice sheath blight pathogen, *R. solani* (Yang et al., 2017). The production of cell wall degrading enzymes such as (cellulase, chitinolytic and polygalacturonase) and antifungal secondary metabolites (siderophore) are common mechanisms that CD-based bacteria use to inhibit the growth of fungal pathogens (Swain et al., 2008).

Nedunchezhiyan et al. (2011) developed an eco-friendly technology comprising common salt (NaCl) solution (1000 ppm), cow urine, CD slurry (2 kg of CD in 1 L of water) in reducing elephant foot yams (*Amorphophallus paeoniifolius*) corm damage by mealybugs (*Rhizoecus amorphophalli*).

4.2. Growth promotion

IAA and gibberelic acid are two important phytohormones that coordinate growth and development in plants. Production of IAA from Gram-positive bacterium, *B. amyloliquefaciens* FZB42 (Idris et al., 2007) and other bacillus species (i.e., *Bacillus safensis, B. cereus, B. subtilis, Lysinibacillus xylanilyticus* and *B. licheniformis*) (Swain et al., 2007; Radha and Rao, 2014) isolated from CD, were reported. In India, farmers apply

Table 1
Recent investigations on biotechnological application of cow dung and/or cow dung microflora.

Biotechnological property	Fermentation type/experiment (s) involved	Major findings	Yield (unit)/ Energetic quality	References
Enzymes				
Bacillus sp.	SmF	CMCase	0.0036 μmolmg^{-1}min^{-1}	Das et al., 2010
Bacillus subtilis CM5	SSF	Exo-PG	229.0 U/gds	Swain and Ray, 2010
Bacillus subtilis VV	SSF	Protease	152.61 U/mg	Vijayaraghavan et al., 2012a
Halomonas sp. PV1	SSF	Protease	1351 U/g	Vijayaraghavan et al., 2012b
Pseudoalteromonas sp. IND11	SSF	Fibrinolytic enzyme	1573 U/ml	Vijayaraghavan et al., 2014
Bacillus cereus IND4	SSF	Amylase	464 U/ml	Vijayaraghavan et al., 2015
Bacillus subtilis IND19	SSF	CMCase; Protease	CMCase: 497.4 U/g; Protease: 4778.2 U/g	Vijayaraghavan et al., 2016a
Bacillus halodurans IND18	SSF	CMCase	4210 IU/g	Vijayaraghavan et al., 2016b
Methane yield				
Cattle manure-food waste	Anaerobic co-digestion	CH_4	406 L/kg -VS	El-Mashad and Zhang, 2010
CD	Anaerobic digestion	CH_4	201 L/kg -VS	Ashekuzzaman and Poulsen, 2011
Cattle manure-food waste-sewage sludge	Anaerobic co-digestion	CH_4	603 LCH_4/kg VS$_{feed}$	Maranón et al., 2012
CD- biomethanisation	Anaerobic digestion	CH_4	26.478 m3 of biogas for 77 days	Ounnar et al., 2012
Cattle manure-organic kitchen waste	Anaerobic co-digestion	CH_4	14,653.5 ml/g-VS	Aragaw and Gessesse, 2013
Cattle manure-food waste	Anaerobic co-digestion	CH_4	388 mL/g-VS	Zhang et al., 2013
Cattle manure-food waste	Anaerobic co-digestion	CH_4	1.40–1.53 L CH_4/LR/d	Agyeman and Tao, 2014
Hydrogen production				
Clostridium cellulosi	Dry fermentation	H_2	743 ml-H_2/kg-cow dung	Yokoyama et al., 2007
Clostridium stercorarium subsp. *leptospartum*.	Dark fermentation	H_2	0.44 mol-H_2 /mol-hexose	Nissila et al., 2011
Cow manure slurry	Semi-CSTR	H_2	10.25 ± 4.96 ml-H_2/g-VS	Wang et al., 2013
Biosorption and Bioremediation				
CD and poultry manure	Dilution plate count method	*Pseudomonas* and *Bacillus* spp	Crude oil degradative capabilities	Akinde and Obire, 2008
DCP	Batch biosorption experiments	AC of 10.20 mg/g; $\Delta G^\circ = -2.837$ kJ/mol, $\Delta H^\circ = -4.757$ kJ/mol and $\Delta S^\circ = 16.64$ J/mol K	Increased biosorption of Cr (VI)	Barot and Bagla, 2012
Proteus vulgaris strain CPY1 and *Pseudomonas aeruginosa* strain LPY1	Batch culture experiment	Actinomycete bacteria	Potential degradation of pyrene	Adebusoye et al., 2015
CD as reductant	Anaerobic digestion	Heat and reducing gases (CO and H_2)	Reduction roasting of low grade iron ore	Rath et al., 2016

AC: Adsorption capacity; **CMCase**: Carboxymethyl cellulase; **DCP**: Dry cow dung powder; **Exo-PG**: Exo-polygalacturonase; **LR**: Loading rate; **MHC**: Moisture holding capacity; **PUB**: Petroleum utilizing bacteria; **Semi-CSTR**: Semi-continuously stirred tank reactor; **SSF**: Solid state fermentation; **VS**: Volatile soilds.

CD traditionally on yam tubers before planting with the traditional belief that it would promote sprouting and seedling growth and prevent seedling rotting (Swain and Ray, 2009a). Swain et al. (2007) demonstrated the production of IAA *in vitro* by *B. subtilis* strains (CM1-CM5), CD isolates. Further, the extraneous application of *B. subtilis* culture suspension and/or CD slurry on yam minisetts increased the number of sprouts, roots and shoots length, root and shoot fresh weights and root: shoot ratio over those minisetts not treated with CD slurry or *B. subtilis* suspension (Swain et al., 2007). Soil amended with *Panchagavya* at concentration of 1:100 (*Panchagavya:* soil, v/w) increased both shoot and root growth of the seedlings of pulses, *Vigna radiata, Vigna mungo, Arachis hypogea, Cyamopsis tetragonoloba, Lablab purpureus, Cicer arietinum* and the cereal, *Oryza sativa* var. *ponni* (Sangeetha and Thevanathan, 2010). Likewise, the application of *Panchagavya* recorded higher growth and yield of black gram than NPK- and untreated control (Kumar et al., 2011). Vijayakumari et al. (2012) investigated the effect of *Panchagavya*, humic acid and micro- herbal fertilizer on the yield of Soya bean (*Glycine max* L.). The maximum pods, number of seeds, protein and ascorbic acid content of the harvested seeds were significantly higher in combined inoculation of *Panchagavya*, humic acid and micro-herbal fertilizer than the individual treatment. *Panchagavya* was found to exhibit a higher population of total bacteria, actinomycetes, P solubilizers, and fluorescent *Pseudomonas* than the control (Amalraj et al., 2013). Moreover, dehydrogenase activity and microbial biomass carbon were found to be higher in *Panchagavya*. The seeds of pigeon pea *(Cajanus cajan* L.*)* were treated with *Panchagavya* that showed enhanced growth of roots and shoots, leaf area, chlorophyll content and photosynthetic activity after 15 days of sowing.

4.3. Biochar

'Biochar' was prepared from dry cow manure pyrolized (CD after thermal treatment/pyrolysis obtained organic fertilizer) at 500 °C. The application of biochar at 20 t/ha mixing rates (with sandy soil) increased maize grain yield by 98% as compared with treatment with no biochar (Uzoma et al., 2011).

4.4. Phosphorus(P) solubilization and zinc mobilization

Some of the microorganisms that reside in CD possess acid and alkaline phosphatase activity that bring about the transformation of insoluble forms of P into soluble forms (Walpola and Yoon, 2012). These P-solubilizing microorganisms include a wide range of bacteria, fungi, and actinomycetes, many of which are common in the rhizosphere (Swain et al., 2012; Radha and Rao, 2014).

Zinc deficiency is a major problem leading to improper plant growth and degradation of soil quality. The cow dung inhabiting bacteria mobilize insoluble form of Zn in soil, making them easily available for plants

(Bhatt and Maheswari 2019). Among the bacteria examined, *Bacillus megaterium* could be exploited for factors such as nutrient management of Zn, growth promotion of *Capsicum annuum* L., and Zn augmentation in soil.

4.5. Sulfur (S) oxidation

A wide variety of CD microflora is involved in S oxidation, in which *Thiobacillus* group of bacteria is the most important and common S-oxidizing agent (Swain and Ray, 2009a). Other microorganisms as S-oxidizer reported include *Bacillus* sp., *Klebsiella* sp., *Pseudomonas* sp. (Devi et al., 2016) Biomass, organic amendments and CD organic carbon content have been related to S oxidation rates. The addition of S to organic CD carbon stimulates S oxidation (Okabe et al., 2010).

4.6. Organic farming/CD fermentation

Because of the growing awareness about eco-friendly organic farming and biotechnology, natural sources such as CD have been used to produce vermicompost with enhanced growth-promoting effects in the crops (Ali et al., 2015). Yadav et al. (2013) produced vermicompost from CD and developed biogas plant slurry under field conditions. Nattudurai et al. (2014) studied the vermicomposting of coir-pith with CD by earthworm, *Eudrilus eugeniae* and observed that it enhanced the growth of *Cyamopsis tetragonaloba*.

Indigenous formulations based on CD fermentation are the source of inoculums of beneficial microorganisms and are commonly used in organic farming. Radha and Rao (2014) reported biodynamic preparation of *Panchagavya* and cow pat pit. These preparations noted a high amount of macro-and micro-nutrients, growth-promoting substances like IAA, gibberellins, and beneficial microorganisms. The beneficial microorganisms showed high counts of *Lactobacilli* (10^9 /ml) and yeasts (10^4 /ml).

4.7. Biocomposting- covering thermophilic bacteria and actinomycetes

Microbial population changes in the level of mesophilic and thermophilic fungi and actinomycetes were studied during composting of CD (Godden et al., 1983; Rahman et al., 2014). Compost extract contains a high population of microbiota such as *Rhizobacteria, Trichoderma*, and *Pseudomonas* sp. that enhances growth and yield of crops (Hirzel et al., 2012). These microbiota produce plant growth hormones and chemical compounds such as siderophores, tannins, and phenols that are antagonistic to various soil pathogens (Mehta et al., 2014). Other microbiota, caused benefit to plants through mechanisms of N_2- fixation and P solubilization (Mehta et al., 2014). The use of compost extract is also claimed to increase soil Carbon levels, improve soil structure, nutrient cycling and water holding capacity, and suppress plant diseases (Shrestha et al., 2011).

4.8. CD-based bioformulations

Kolandasamy and Ponnusamy (2011) patented a bioformulation process of plant growth-promoting rhizobacterium (PGPR) for biocontrol of red rot root diseases. The PGPR bioformulation consisting *Pseudomonas fluorescens* VP5 (isolated from tea rhizosphere) immobilized with vermicompost as well as CD) synthesizes antibiotic compounds claimed active against the pytopathogen *Poria hypolateritia* and effectively inhibited root pathogen. Three CD-based biodynamic preparations, i.e., Panchagavya \(PG), BD500 and 'Cowpat pit' (CPP) were developed dominated by *Bacillus* spp. that exhibited plant growth promoting attributes like indole 3- acetic acid production, phosphate solubilization, antagonism to *Rhizoctonia bataticola* and improved growth of maize plants (Radha and Rao, 2014).

4.9. Significance of CD applications in agriculture in context to climate change

PGPR play a pivotal role in the sustainable agriculture system (Bhattacharyya and Jha, 2012; Glick, 2012). For decades, the most important PGPR commercialized, belong to the genera of *Pseudomonas, Bacillus, Enterobacter, Klebsisella, Azotobacter, Variovorax, Azosprillum*, and *Serratia* (Glick, 2012; Ahemad and Kibret, 2014).

The microbiota of CD and PGPR have similar attributes, i.e. both promote plant growth by regulating nutritional and hormonal balance, produce plant growth regulators/phytohormones (IAA, cytokinin, Gibberellin, kinetin), solubilize nutrients (P and S) and provide resistance against plant pathogens (Siddiqui and Futai, 2009; Ray and Swain, 2013). However, microflora from CD has advantages over PGPR due to its potential to tolerate heat, UV radiation and oxidizing agents (Ray and Swain, 2013). Moreover, CD microflora produces hyperthermostable enzymes (Swain et al., 2007, 2009a), since rumen bolus temperatures vary from 39.5 ˚C to 40.3 ˚C due to the activity of heat-producing rumen microorganisms (Bodas et al., 2014) that is normally higher than atmospheric temperature (Timsit et al., 2011). In the context of these advantages of CD microflora encourage exploiting their applications as biofertilizer in tropical agriculture in context to climate change (Swain et al., 2012).

4.10. Pond productivity and fish growth

In aquaculture, fish productivity and fish growth are influenced by two major factors, nutrient input and fertilization and the pond management practices (Verdegem, 2013). A significant correlation has been noticed within fertilization of CD on different fish pond parameters, such as dissolved oxygen (DO), biochemical oxygen demand (BOD), alkalinity, nutrient release, net primary productivity (NPP), plankton density (no./L), fish growth/biomass and specific growth rate (SGR) of fish in pond productivity (Gandhi, 2012). CD application increases pond productivity in terms of plankton production and builds up fish biomass/growth (Garg and Bhatnagar, 1999). The fertilization of the pond with raw CD increases the alkalinity, plankton population and densities that further regulates primary and optimal productivity of the fish pond (Singh et al., 2010). A manuring rate of 10,000 kg/ha CD was found optimum in pond productivity along with inorganic fertilizers, single super phosphate (Garg and Bhatnagar, 1999). More recently, Kaur and Ansal (2010) reported that the production and growth of exotic carp (*Cyprinus carpio* L.) were increased with the utilization of semi-digested CD at a dose of 20,000 kg/ha/year. The maximum growth and fish yield by application of CD can be attributed to higher zooplankton production and superior water quality in terms of high DO values (Godara et al., 2015).

5. Biotechnological applications

The CD is a veritable multipurpose commodity considered as a natural phytoprotectant that may be biotechnologically exploited in various ways (Fig. 3). CD is of special biotechnological interest since their inhabitant microorganisms are thermotolerant and produce an array of biocommodities (Table 2).

5.1. Microbial enzymes

Microbial enzymes have extensive applications in pulp, paper, textile, food and beverage industries (Behera and Ray, 2016; Panda et al., 2016). The distinct clade of microorganisms in CD holds some of the resilient species capable of growing in extreme environments (Panda et al., 2016). In a recent study, *Streptomyces* spp. isolated from cow faces were found to produce an array of industrially important enzymes such as amylase, caseinase, gelatinase, lipase, chitinase and cellulase (Semwal et al., 2018).

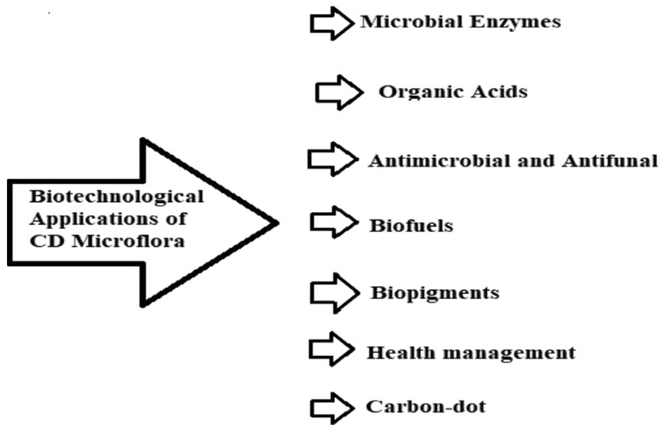

Fig. 3. Biotechnological applications of cow dung microflora.

5.1.1. *Enzymes from CD microorganisms*

α-Amylase: **α-** Amylases have various applications in food, fermentation and pharmaceutical industries (Ray et al., 2008; Panda et al., 2016). In an earlier investigation, Obi and Odibo (1984) reported a neutral and thermo-stable *α*-amylase (optimum activity at pH 7 and 80 °C, respectively) from *Thermoactinomyces* sp. isolated from CD. Swain et al. (2006) reported production of a thermostable *α*-amylase by *B. subtilis* CM3 (isolated from CD), having a molecular mass of 18 ± 1 kDa with optimum activity: temperature, 50–70 °C; pH, 5–9; growth in a wide range of N and C sources, a trait that the strain can be incorporated into cattle feed for compatibility with the gut environment and easy digestibility.

Carboxymethyl cellulase: CMCase is widely used in bioenergy, detergent, textile, food, and paper industry and the global sale was $4.4 billion in 2015 (Vijayaraghavan et al., 2016a,b). Mainly, thermotolerant *Bacillus* spp. from CD were reported to produce CMCase (Das et al., 2010; Sadhu et al., 2014)

Exo-polygalacturonase: Swain and Ray (2010) reported the exo-polygalacturonase production by *B. subtilis* CM5 isolated from CD, which was comparable to marketed pectinase (Pectinex®, Novozyme, Denmark). Application of *B. subtilis* crude exo-PG resulted in 13.3% increase in yield of carrot juice in comparison to the juice extracted with commercial Pectinex (Novozyme, Denmark). The optimum parameters for exo-polygalacturonase production(82.0–83.2 units) were: temperature (50 °C), pH(7.0) and incubation period (36 h).

Mutlienzyme complex: Bacillus species continue to play a significant role in microbial fermentation (Schallmey et al., 2004). During the search of xylan-degrading microorganisms, Velazquez et al. (2004) reported a novel sporulated bacterial genus *Paenibacillus* (Family: Paenibacillaceae) from CD. This species produced a wide range of hydrolytic enzymes, i.e., amylases, cellulases, *β*-glucosidase, urease and xylanases activity.

5.1.2. *CD as substrates for enzymes*

CD is often considered as good low-cost substrate for microbial enzyme production in solid-state fermentation (Mukherjee et al., 2008). Vijayaraghavan and Vincent (2012a) produced a halo-tolerant alkaline protease by *Halomanas* spp. PV1 using CD as semi-solid substrate. As compared with wheat bran (1013 U/g), CD supported the maximum protease production (1351 Units/g) at the following optimum process parameters: the fermentation period (72 h); pH (8.0); initial moisture content (140%, v/w) and the inoculum level (15%, v/w). The same group reported production of several enzymes using CD as the substrate, i.e. fibrinolytic enzyme by *Bacillus* sp. IND7 (Vijayaraghavan et al., 2016b) and *Pseudoalteromonas* sp. IND11 (Vijayaraghavan and Vincent (2014), CMCase and protease by *B. subtilis* IND19 (Vijayaraghavan et al., 2012b, 2016a,b), alkaline protease by *Pseudomonas putida* Strain AT (Vijayaraghavan et al., al.,2014) and amylase by *B. cereus* IND4 (Vijayaraghavan et al., 2015).

5.2. *Organic acids*

Lactic acid is produced by a mixed culture of lactic acid bacteria isolated from CD (Gómez-Hernández and Vega, 1982).

Cow dung was used as feedstock for the production of a high value-added chemical levulinic acid in dilute acid aqueous solutions. A high levulinic acid yield of 338.9 g/kg was obtained from the pretreated cow dung, which was much higher than that obtained from the crude cow dung (135 g/kg), mainly attributed to the breakage of the lignin fraction in the lignocellulose structure of the cow dung by potassium hydroxide (KOH) pretreatment (Su et al., 2017).

5.3. *Antimicrobial and antifungal activity*

The development of antibiotics from agricultural products impacts the treatment of diseases affecting the human population (Rahimi and Nayebpour, 2012).Teo and Teoh (2013) reported CD acts as antibacterial agents against several Gram-positive, i.e., *B. subtilis, B. cereus, B. sphaericus, Enterococcus faecalis, Staphylococcus epidermidis, Staphylococcus aureus, Micrococcus luteus* and Gram-negative, i.e., *E. coli, Pseudomonas aeruginosa, Proteus vulgaris* and *Salmonella* bacteria. Shrivastava et al. (2014) evaluated the antimicrobial and antifungal properties of CD extract against *Candida, E. coli, Pseudomonas* and *Staphylococcus aureus* and found it highly effective against these microbes. Lu et al. (2014) isolated 209 bacterial strains from CD. Among these, 59 isolates (genera *Proteus, Providencia* and *Staphylococcus*) displayed nematicidal activity against the nematode *Caenorhabditis elegans*. However, 14 strains showed nematicidal activity against pathogenic nematode, *Meloidogyne incognita*. Evaporated extract of CD was found to possess antimicrobial activity against bacteria, *S. aureus* (Lu et al., 2014). Several observations suggested that antimicrobial peptides from CD microflora can disrupt the integrity of the cell membrane and surface permeability, and thus prevents the nutrient uptake, and induces pore formation that kills the bacterial cells (Fjell et al., 2012).

In a recent study, bacteriocin producing lactic acid bacteria were isolated from CD were found to control the growth of post-harvest spoilage microorganisms of fruits such as *E. coli, S. aureus, B. cereus, P. aeroginosa, Proteus vulgaris, Salmonella* Typhi, *Serritia* spp., *Xanthomonas campestris*, and also against *Aspergillus flavus, A. niger, Fusarium, Alternaria, Saccharomyces cerevisiae* using the diffusion bioassay plate method (Dhundale et al., 2018). Thus, the shelf-life of fruit can be extended with the application of these lactic acid bacteria in immobilized coatings.

5.4. *Alternative fuels (bio-energy)*

There are a large number of reports on beneficial applications of CD on biogas and bio-hydrogen production (El-Mashad and Zhang, 2010; Membere et al., 2012).

5.4.1. *Biogas*

The potential of biogas as an important source of energy stands in second position, next to solar energy systems (Panwar et al., 2011). The constituents of biogas include methane (CH_4), as the primary constituent and other gases such as CO_2, H_2S, NO, SO, etc., as secondary constituents (Ward et al., 2006; Singh and Sankarlal, 2015).

CD being rich in methane content is extensively used as organic agricultural fertilizers as well as for production of biogas for ages (Teo and Teoh, 2013). The organic matter in CD is largely decomposed by the actions of cellulolytic bacteria present in it (Gashaw, 2016). The anaerobic conditions leading to the production of biogas comprise three stages: hydrolysis, acidogenesis/acetogenesis and methanogenesis. The methanogenesis bacteria acted upon the organic matter in anaerobic conditions

Table 2
Agricultural significance of cow dung formulation and/or cow dung microflora.

Agricultural importance	Microflora/enzyme/gene/ special property involved	Method (s) involved	Applications	References
Biological control				
Combitorial effect of CD and CU	NR	Spore germination/MGI test/*In vitro* activity	Prevents *Fusarium* wilt of cucumber	Basak et al., 2002
Combitorial effect of plant extracts, CD and CU	NR	Spore inhibition test	Inhibition (91%) of conidial germination	Akhter et al., 2006
CD with *in vitro* growth of fungi	*B. subtilis* strains CM1 and CM 3/ amylase and cellulase	Antagonism study of dual-culture plate method	Prevents from rots of yam (*Dioscorea rotundata*) tubers	Swain and Ray, 2009a
CD-mediated wilt in chickpea	Chitinase/Pectatelyase/Cellulase	*In vitro* assay of fungal inhibition	Reduction of wilt in *C. arietinum*	Nautiyal et al., 2013
CD on phytopathogenic fungi	Extracellular enzymes	MGI test/*In vitro* study	Potential to control against red rot disease in sugarcane	Patel et al., 2016
Vermicompost	Soil pH, IAA and microbial activity	NR	Increased defense against root-knot nematode (*Meloidogyne incognita*) in tomato plants	Xiao et al., 2016
CD with field application	*Streptomyces cochorusii* NF0919 and *Bacillus amyloliquefaciens* SB177	MGI test/*In vitro* study	Potential agent against rice sheath blight pathogen	Yang et al., 2017
Growth stimulation				
IAA	*Bacillus subtilis* strains (CM1-CM5)	Extraction and bioassay of growth regulators	Promoted sprouting of tuber (yam)	Swain et al., 2007
Seed germination	NR	Germination study/ seeds on 2 - 3% Panchagavya treatment	Increased the growth of greengram [*Vigna radiata* (L.) plant	Kumaravelu and Kadamban, 2009
Panchagavya, vermicompost and FYM	Bacteria, actinomycetes, phosphate solubilizers, nitrifiers	NR	Promoted growth pigeon pea (*Cajanus cajan* L.)	Amalraj et al., 2013
Microbes and organic manure (CD)	Rhizospheric bacteria and mycorrhizal fungi	Glass house and field conditions	Bio-inoculants improved *Ocimum basilicum* growth under salinity stress	Bharti et al., 2016
Vermicompost and probiotics	Bacillus megaterium BM and Bacillus amyloliquefaciens BA	NR	Increased the yield, soluble sugar and protein contents of Tomato	Fei et al., 2016
Organic farming/CD fermentation				
Anaerobic digestion/ balloon digester	Specific microbes in DCM	Viable plate count assay	Reduced bacterial pathogen count (DL = 10^2 cfu/g manure)	Manyi-Loh et al., 2014
Vermicomposting	Worms and associated microbes	BPS mixing with CD using *Eudrilus eugeniae*	Management of SW/ increase in NPK value	Rajeshkumar and Ravichandran, 2015
Vermicomposting of SPW	Cellulolytic microbial population and cellulase activity	NR	Enhanced decomposition of SPW	Pramanik et al., 2016
Bioformulation				
Vermicompost-based (granular and its aqueous extract)	*Rhizobium meliloti*	Water-holding capacity	Growth promoter	Kalra et al., 2010
Vermicompost-PGPR	PGPR	NR	Improves soil quality and crop yield	Song et al., 2015
Soil fertigation				
CMB	Favoured maximum P availability	Greenhouse experiment	Improved the physio-chemical properties of the coarse soil	Uzoma et al., 2011
Soil amendment/ P solubilization	Organic fertilizers	10% (w/w) CD, biogas slurry and vermicompost with soil	Increased MPAC of soil	Seafatullah et al., 2015

BPS: Biogas plant slurry; **CMB**: Cow manure biochar; **CU:** Cow urine; **DCM**: Dairy Cattle Manure; **DL**: Detection limit; **IAA:** Indole-3-acetic acid; **MGI**: Mycellial growth inhibition; **NR**: Not reported; **MPAC**: Maximum phosphorus adsorption capacity; **PGPR**: plant growth promoting rhizobateria; **SPW**: Shredded pruning wastes; FYM: Farmyard manure; **SW**: Solid wastes.

(Gashaw, 2016). Ounnar et al. (2012) developed a laboratory experiment of mesophilic anaerobic digester (800 L capacity) of CD (440 kg) as organic waste that gave biogas production of 26.478 m^3 with an average optimal composition of 61% in the methane of energy equivalent of 592.8 MJ (164.5 kWh). Tewelde et al. (2012) observed the biogas production from the anaerobic co-digestion of brewery and CD in a proportion (70:30) in batch mode at mesophilic conditions. The average gas (methane) yield was found to be 0.290 m^3/kg. The mixtures of pig manure and CD in various proportions provide a better nutrient balance and consequently, higher biogas yields. Li et al. (2014a) evaluated the dry anaerobic digestions (at 35 °C) of CD mixed with pig manure in different ratios in a single-stage batch reactor. The dry co-digestion of 60%

CD and 40% pig manure delivered the highest methane yield. Singh and Sankarlal (2015) investigated the generation of biogas using a mixture of kitchen waste and cow manure in anaerobic digesters. A temperature range of 30–35 °C is maintained to facilitate the mesophilic conditions. The amount of biogas (0.05196 m^3) was produced with CH_4 content of about 60 percent. More recently, Resende et al. (2016) studied the diversity and composition of microbial structure in pilot-scale anaerobic digestion of CD for production of methane, operating at ambient temperature. The result suggested that redundancy of microbial groups has occurred in a complex microbial community at ambient temperature systems for methane production (Abdeshahian et al., 2016).

5.4.2. Bio-hydrogen

Anaerobic fermentation with CD microorganisms to produce biohydrogen has been well documented. Based on constituents of fermentation products, three types of fermentation processes: (1) propionic-type, (2) butyric-type, (3) and ethanol-type, are defined (Fan et al., 2006). In propionic-type fermentation, propionic and acetic acids but no hydrogen is produced (Sinha and Pandey, 2011). However, in butyric acid fermentation, H_2, CO_2, butyric and acetic acids are the prime products. The ethanol-type fermentation results in the formation of H_2, CO_2, ethanol and acetic acid (Ren et al., 2010). Fermentation of organic wastes, such as animal and food wastes is a potential renewable source of energy (Yokoyama et al., 2007). Rumen fluid can enrich thermophilic, cellulolytic and hydrogen-producing microorganisms (Nissila et al., 2011). Yokoyama et al. (2007) studied dry hydrogen fermentation (without dilution) of CD (15% Total Solids) in laboratory-scale batch experiments. The dry-fermentation produced 743 ml H_2/kg CD at an optimum temperature of 60 °C with butyrate and acetate formation (Nissila et al., 2011). Ren et al. (2010) reported that CD compost-enriched cultures were ideal microflora for hydrogen production from cellulose. In the anaerobic fermentation process, the carbon/nitrogen (C/N) ratio is a critical factor representing nutrient balance in the medium for biohydrogen production (Wagner et al., 2012). Li et al. (2014b) co-digested the CD compost with glucose and apple pomace in batch fermentation and investigated the effects of C/N ratio on biohydrogen production. The addition of CD in the anaerobic co-digestion process enhanced the buffer capacity (created by NH_4^+ and volatile fatty acids allowing high organic load without pH control (Zhang et al., 2013). In a recent study, biohydrogen was obtained from a mixture of CD and food waste (1:1 ratio) done by dark fermentation and the composition of gas produced was determined using gas chromatography which confirmed the presence of biohydrogen of 26.9% yield (Antony et al., 2018)

5.4.3. Perspective of biogas production in Asian countries

The 19th livestock census shows the total population of cattle in India is 190.90 million; out of which 151.17 million are indigenous and 39.73 million are cross-breed or exotic (Balamurugan et al., 2012). In India, nearly 70% of the human population resides in villages, where the cow is the major cattle and generates 9–15 kg dung/cow/day (Yadav et al., 2013). Thus, it is presumed that the total population of cows (9–15 kg x 190.90 million) approximately generates 1718.1×10^3 to 2863.5×10^3 tons dung/day. It has been postulated that CD generated from 3 to 5 cattle/day can run a simple 8–10 m^3 biogas plant which can produce 1.5–2 m^3 biogas/day (Gupta et al., 2016). The total amount of dung can produce 286.35×10^3 m^3- 381.8×10^3 m^3 biogas/day. The total sum can support 191, 000–255, 000 families (at least 6 persons/family) for domestic cooking of food (2 times)/ day.

A study of biogas production in Malaysia from farm animal waste (cattle dung) in the year 2012 showed that biogas potential of 4589.5 million m^3/year could be produced from cattle dung that could provide an energy generation of 8.27×10^9 kWh/year (Abdeshahian et al., 2016). Halder et al. (2016) studied the production potential of domestic biogas from livestock manure and agricultural residues in rural Bangladesh. From the total residues of 106.27 million tons, 63,78 million tons were from livestock (cattle dung) that can generate potentially2.6 billion m^3 of biogas. More recently, biogas technology has been adopted in Africa, where a dire energy crisis currently prevails (Roopnarain and Adeleke, 2017). Cow dung–urine biorefinery as a representative biomass processing enterprise was assessed for economic, environmental and social sustainability parameters (Jogelkar et al., 2020).

5.5. Biopigment production

Mondal et al. (2015) studied the total aerobic heterotrophic bacteria of CD. Out of 15 bacterial isolates, CD 5 showed deep red pigmentation in a nutrient broth culture medium that had similarities with Rhodamine-6 G. The potential CD 5 bacterial isolate was confirmed by 16 s rRNA gene sequencing and found to belong to the genus, *Bacillus*. More recently, on phylogenetic analysis (16Sµ DNA sequencing), pigmented bacteria (CD 7) were identified as *Pseudomonas* (Malik et al., 2016).

5.6. Human health management

Immunomodulatory, immunostimulatory and anti-inflammatory effects of *Panchagavya* are mentioned in *Ayurveda* (Dhama et al., 2005; 2014). Fresh CD, apart from antifungal properties (Patulodin-like compounds, CK2108A and CK2801B) (Tuthill and Frisvad, 2002) are found to kill the germs of malaria and tuberculosis (Khan et al., 2015).

5.7. Carbon-dot

Carbon nanodots (CNDs) which are part family of carbon nanoparticles have drawn a lot of attention due to their prominent characters and wide prospective applications. The materials are nontoxic and exhibit fluorescence properties that are potential for application in photocatalysis, optoelectronic, bioimaging and sensors (Haryadi et al., 2018).

CD serves as a low-cost substrate for carbon-dot synthesis (Haryadi et al., 2018; Ramalingam et al., 2020). Carbon-dots were synthesized from cow manure which was used for cellular selectivity for nucleoli staining. The synthesized Carbon-dots were modified by functionalizing (amine-passivated) with ethylenediamine, affording amide bonds that resulted in bright green fluorescence. The new modified C-dots were successfully applied as selective live-cell fluorescence imaging probes with impressive subcellular selectivity and the ability to selectively stain nucleoli in breast cancer cell lineages (MCF-7) (D'Angelis Do ES et al., 2015).

6. Environmental applications

Traditional uses of CD in Asian households as burning for fuel purposes or cooking causes greenhouse gas emission. The best alternative is to valorize it for the production of biogas, biofertilizer and bioelectricity.CD has been used also in several other applications concerning environmental issues such as xenobiotics degradation, bioremediation, and as bioabsorbent.

6.1. Biosorption, bioremediation and biodegradation

CD is recognized as an eco-friendly and indigenous material for biosorption (removal) of heavy metal ions (Wang and Chen, 2009; Geetha and Fulekar, 2013; Gupta et al., 2016). Barot and Bagla (2012) reported the application of dry CD powder in removing Cr (VI) from aqua- medium. Rahman et al. (2014) performed a series of batch experiments in presence of fresh CD for the removal of arsenic both from aqueous solution and arsenic-rich wastes. The microorganisms present in CD volatilized arsenic from solution and sludge. The bioleaching of Pb (64%) and Cu(49%) was reported after 54 h of incubation with *Panchagavya* (Praburaman et al., 2015). High-performance chromatography analysis showed the presence of lactic, malic, acetic, citric, and succinic acids in *Panchagavya* that may be the key factors in the removal of heavy metals from the contaminated soil. More recently, the CD was used as a reductant in the reduction roasting and magnetic separation of complex and low-grade iron ore slime (containing 56.2% Fe). The generation of heat from CD (organic volatile cake) and the possible generation of reducing gases (CO and H_2) from the combustion of the hydrocarbon content of CD has a potential role in the reduction process (Rath et al., 2016).

The potential of CD microflora for bioremediation of hazardous compounds, i.e. benzene, toluene, xylene, phenol, and halogenated compounds has been documented (Singh and Fulekar, 2009).

Singh and Fulekar (2010) investigated biodegradation of benzene by *Pseudomonas putida* MHF 7109 isolated from CD in bioreactor. *P. putida* MHF 7109 strain was reported to contain degrading enzymes like oxidase (cytochromeoxidase) and catalase which may help in effective degradation of benzene to nearly 68% within 12–68 h of treatment. Likewise, *Bacillus* sp., isolated from CD was found to be effective for degradation of halogenated compound (2, 2-dichloropropionic acid) (Smail, 2014). Currently, the metabolic functions of microorganisms are being challenged by unquantifiable amounts of xenobiotics released into the environment. Adebusoye et al. (2015) reported pyrene detoxification by *Proteus vulgaris* strain CPY1 and *P. aeruginosa* strain LPY1 from CD.

6.2. Biofiltration technology

For the removal of ammoniacal compounds, biofiltration technology is used (Rattanapan and Ounsaneha, 2011). Several reports have been noted that mature compost from cattle manure acts as an important candidate for biofilter medium. Kitamura et al. (2016) investigated the bacterial community profile and the chemical constituents of the compost from different compost of food waste and cattle manure. As compared to food waste compost, the cattle manure composts showed a greater alpha diversity (species diversity) of bacterial communities. The diversity of local species was found in abundance with rRNA gene fragments and ammonia monooxygenase (*amoA*) genes and the presence of nitrifying bacteria such as proteobacteria were inhabited with it. The result suggested that the compost made from cattle manure is more suitable for the biofiltration of foul-smelling substances like ammonia.

6.3. Bioadsorbent

The presence of heavy metals (e.g., Zn, Cu, Pb, Ni, Cd, etc.) in wastewater and industrial effluents constitutes a major environmental problem. CD ash is an eco-friendly and low-cost absorbent that contains 12.48% calcium oxide, 0.9% magnesium oxide, 0.312% calcium sulfate, 20% aluminum oxide, 20% ferric oxide and 61% silica (Vasanthakumarn and Bhagavanalu, 2003). The presence of a maximum percentage of silica exhibits considerable affinity for metal ions (Qian et al., 2008). Thus, the CD could be efficiently used as a promising adsorbent in the removal of heavy metals from wastewaters and the environment (Ojedokun and Bello 2016; Mandavgane and Kulkarni, 2020).

CD is also found to adsorb textile dyes like Methylene blue, Blue RGB, and Eosin YWS from the wastewater (Rattan et al., al.,2008). CD ash could reduce 66% COD (Chemical Oxygen Demand) of wastewater (Kaur et al. (2016).

6.4. Miscellaneous compounds

6.4.1. Silica

Biomass ashes including CD are a rich source of silica, Silica from CD ash was extracted by alkali digestion and acid precipitation method. CD ash was calcinated at 630 ˚C before alkali digestion at 100 ˚C for 3 h. The digested solution was acid washed to precipitate amorphous silica having 200 nm particle size and very high purity (Sivakumar and Amutha, 2018).

CD ash is found as a supplementary material to mortar and concrete by replacing Portland cement up to 30% (Rayaprolu and Raju, 2012).

6.4.2. CD- Poly(lactic acid) (PLA) blending

CD (average 4 mm in size) was reinforced in poly(lactic acid) (PLA) biocomposites for potential use in the load-bearing application. The results showed an improvement in the flexural properties, while the tensile and impact strength dropped by 20 and 28% with the addition of 50% CD. The decline in the tensile and impact strength was due to microcracking and voids formation at higher CD content (Yusefi et al., 2018). SEM analysis of tensile and impact fractured surfaces indicated that the CD had a reasonable adhesion with the matrix. Moreover, the SEM micrographs of soil burial studies showed an accelerated degradation of higher CD wt% biocomposites.

7. Patents and innovations

In the last 10 years, there is a surge of publications, innovative technologies and patents coming out from the valorization of CD and cow urine. We are citing here some of the patents on CD-based technology.

7.1. Patents

- **Method for treating cow dung with *Hermetia illucens* to prepare organic fertilizer**. The invention provides a method for treating CD with *Hermetia illucens* to prepare an organic fertilizer (Patent application of CN104844288A/en).
- **Preparation method of nano cow dung fertilizer**. The method describes the mixing of CD with nano $CaCO_3$, nano TiO_2 and nano-carbon (Patent application of 2014–05–07 CN103772007A).
- **Cow dung and toxic cake biological feedstuff and its preparation**. The invention relates to cow dung and toxic cake biological feedstuff and its preparation, wherein the fodder comprises cattle manure 4000–6000 containing composite microbiological bacterium liquid, cattle manure leaven 10–15, toxic bean cake powder 100–450, the preparation process comprises mixing proportionally, stirring homogeneously, hermetically sealing by compacting in fermentation apparatus, placing under the temperature of 24–28 deg. C, fermenting completely within 4–8 days. The fodder can improve the immunity of various animals and adjust ecological balance in intestinal tract. (Patent application no,2005–09–07 Publication of CN1663420A)
- **Novel cow-dung based microbial fuel cell.** A novel CD-based Microbial Fuel Cell (MFC) comprising of graphite electrodes and a proton exchange membrane and that converts chemical energy available in a bio-convertible substrate directly into electricity and achieves this by using the microorganisms in CD as a catalyst to convert substrate into electrons (Patent application no. US20110135966A1/en).
- **Method for producing fertilizer and grass fiber paper pulp by using cow dung**. The invention relates to a method for producing fertilizer and grass fiber paper pulp by using cow dung. (Patent application 2015–07–01 Publication of CN104744101A
- **Cow dung paper pulp produced with cow dung as material**. The cow dung paper pulp is suitable for producing industrial packing paper or common paper. The present invention provides a new pulp source and has waste utilization, environment friendship, and low cost. (Patent Application CN 101,021,049 A)

7.2. Innovations

There are also many innovative products developed from CD. Few examples are cited here:1. Variety of creations from Mestic (manure)-derived fabrics. https://www.aiche.org/chenected/2016/07/textiles-created-cow-dung; 2. CD is used as feedstock for the production of a high-value-added chemical levulinic acid (LA) in dilute acid aqueous solutions (Su et al., 2017); 3. CD - reinforced (PLA) biocomposites (Yusefi et al., 2018); 4.Extraction of silica from CD (Sivakumar and Amutha, 2018), etc.

8. Zero-waste strategies and circular economy

A zero-waste strategy includes all four of the generally accepted goals of sustainability: value addition, environment protection, improved material flow, and social well being. A zero-waste strategy would use far fewer new raw materials and send no waste to landfills.

The cattle waste is a major source of noxious gases, repugnant odor although it harbors many beneficial microorganisms and other animals like earthworms. Proper utilization of cow urine and dung into biogas, composts and vermicompost, biofertilizer, biogrowth regulator, biopesticides, etc. can be useful to increase crop yield and income in a sustainable agriculture system. The integration of composting and vermicomposting is better compared to either composting or vermicomposting as it requires less time to complete the cycle and the substrate produced after the combined process has better physical and chemical properties which can support crops. The use of CD-based biopesticides protects the environment from the hazardous impacts of the use of chemical pesticides. The recent work on algae cultivation from cattle waste that can be converted into bio-oil and other valuable products support the zero-waste strategy in a circular economy(Sorathiya et al., 2014). The CD-fed integrated fish farming has good potential to generate income. Likewise, the CD has been valorized into a large number of novel products like bioadsorbent, biopigments and construction materials which are eco-friendly, low-cost and useful. Similarly, CD microorganisms have been exploited in biotechnology for the production of enzymes, organic acids, etc., and environmental applications. However, most of these technologies are confined to the laboratory level that needs scale-up and commercialization.

9. Concluding remarks and future prospective

Mining of “omic” technologies (genomics, transcriptomics and proteomics) and enactment of metagenomic libraries and next generation sequencing platforms on CD-microflora can help to unravel powerful functional/novel genes for thermotolearnce and growth regulators /phytohormone production. CD-microflora can serve as probiotics, live microbial food supplements modifying the intestinal microbiota. Another important area of research for future studies is developing microbial enzymes, organic acids, antimicrobials and other biocommodities from CD-isolates for possible applications and mass production. Energetic valorization of biomethane from CD/CD-microflora is required to encourage renewable energy technology as the most appropriate solution for the energy of the future. The process needs to be emphasized to correspond perfectly to the policy of sustainable development.

There is concern that the use of chemical fertilizers and pesticides in agriculture has caused environmental threats. An alternative is to use eco-friendly organic fertilizer (CD) to reduce environmental degradation and pollution. Besides, CD-based fungicides and nematicides can be applied as potential external inputs (organic amendments/microbial inoculants) with the ultimate goal of maximizing productivity and economic returns. CD-based microorganisms are invariably thermotolerants; that attribute can be used in bio-based formulations of fertilizers, microbial enzymes and growth regulators that can help in overcoming the loss of crop productivity in context to climate change. Further research will be carried out to establish stable formulations, interpret the mechanism of the biocontrol agents, and identify the molecular structural formula of secondary metabolites. Moreover, improvement in the scientific understanding by cutting-edge experimentation of CD-based substrate to create more robust and active biocommodities warrants to harvest of diverse agricultural and biotechnological properties of CD-microbiota.

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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