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Review

Distillery spent wash: Treatment technologies and potential applications

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ABSTRACT

Distillery spent wash is the unwanted residual liquid waste generated during alcohol production and pollution caused by it is one of the most critical environmental issue. Despite standards imposed on effluent quality, untreated or partially treated effluent very often finds access to watercourses. The distillery wastewater with its characteristic unpleasant odor poses a serious threat to the water quality in several regions around the globe. The ever-increasing generation of distillery spent wash on the one hand and stringent legislative regulations of its disposal on the other has stimulated the need for developing new technologies to process this effluent efficiently and economically. A number of clean up technologies have been put into practice and novel bioremediation approaches for treatment of distillery spent wash are being worked out. Potential microbial (anaerobic and aerobic) as well as physicochemical processes as feasible remediation technologies to combat environmental pollution are being explored. An emerging field in distillery waste management is exploiting its nutritive potential for production of various high value compounds. This review presents an overview of the pollution problems caused by distillery spent wash, the technologies employed globally for its treatment and its alternative use in various biotechnological sectors.

Contents

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1. Introduction

Distillery spent wash refers to the effluent generated from alcohol distilleries. On an average 8–15 L of effluent is generated for every liter of alcohol produced [1]. The alcohol distilleries are extensively growing due to widespread industrial applications of alcohol such as in pharmaceuticals, food, perfumery, etc. It is also used as an alternate fuel. There are 319 distilleries in India alone, producing 3.25 billion liters of alcohol and generating 40.4 billion liters of wastewaters annually [2]. As per the Ministry of Environment and Forests (MoEF), alcohol distilleries are listed at the top in the "Red Category" industries [3].

Alcohol production in distilleries consists of four main steps viz. feed preparation, fermentation, distillation and packaging [4]. Ethanol can be prepared from various biomass materials but the potential for their use as feedstock depends on the cost, availability, carbohydrate contents and the ease by which they can be converted to alcohol [5]. Nearly 61% of world's ethanol production is from sugar crops [6]. Most Indian distilleries exclusively use cane molasses as raw material for fermentation [7]. Molasses is suitably diluted in order to have desired sucrose level in it. It is then supplemented with assimilable nitrogen source like ammonium sulphate or urea. It is also supplemented with phosphate if necessary. The pH of the fermentation broth is adjusted to below 5 using sulphuric acid. Fermentation is carried out for about 50 h by using 5% active culture of *Saccharomyces cerevisiae*. Ethanol accumulates up to 8–10% in the fermented mash. The fermented mash is then distilled, fractionated and rectified after the removal of yeast sludge [8]. Apart from yeasts, a bacterial strain, *Zymomonas mobilis*, has been demonstrated as a potential candidate for ethanol production [9]. The residue of the fermented mash which comes out as liquid waste is termed as spent wash [8,10,11].

The wastewater generated from distillation of fermented mash is in the temperature range of 70–80 °C, deep brown in color, acidic in nature (low pH), and has high concentration of organic materials and solids. It is a very complex, caramelized and cumbersome agro industrial waste. However, the pollution load of the distillery effluent depends on the quality of molasses, unit operations for processing of molasses and process recovery of alcohols [12].

With government policies on pollution control becoming more and more stringent, distillery industries have been forced to look for more effective treatment technologies. Such technologies would not only be beneficial to environment, but also be cost effective. In 2003, Central Pollution Control Board (CPCB), the national agency responsible for environmental compliance stipulated that, distilleries should achieve zero discharge in inland surface watercourses by the end of 2005 [3]. Consequently, the wastewater needs to undergo extensive treatment in order to meet the stipulated environmental demands.

This review aims to disseminate information about the pollution potential and the strategies implemented for treatment of distillery spent wash. The experiences gained so far and the state of the art technologies are discussed. The potential application of distillery effluent in diverse agro industrial sectors to produce various value added byproducts is also reviewed.

2. Environmental hazards of distillery spent wash

The production and the characteristics of the spent wash are highly variable and dependent on the raw material used and various aspects of the ethanol production process [2,4]. Wash water used to clean the fermenters, cooling water blow down and broiler water blow down further contribute to its variability [2]. Distillery spent wash has very high biological oxygen demand (BOD), chemical oxygen demand (COD) and high BOD/COD ratio. The amount of inorganic substances such as nitrogen, potassium, phosphates, calcium, sulphates is also very high (Table 1). Its recalcitrant nature is due to presence of the brown polymers, melanoidins, which are formed by Maillard amino carbonyl reaction. These compounds have antioxidant properties, which render them toxic to many microorganisms such as those typically present in wastewater treatment processes [15]. The defiance of melanoidins to degradation is apparent from the fact that these compounds escape various stages of wastewater treatment plants and finally enters into the environment. Apart from melanoidins, the other recalcitrant compounds present in the waste are caramel, variety of sugar decomposition products, anthocyanins, tannins and different xenobiotic compounds [12]. The unpleasant odor of the effluent is due to the presence of skatole, indole and other sulphur compounds, which are not effectively decomposed by yeast during distillation [16]. Spent wash disposal into the environment is hazardous and has high pollution potential. High COD, total nitrogen and total phosphate content of the effluent may result in eutrophication of natural water bodies [15]. The highly colored components of the spent wash reduce sunlight penetration in rivers, lakes or lagoons which in turn decrease both photosynthetic activity and dissolved oxygen concentration affecting aquatic life. Kumar et al. [17] evaluated the toxic effect of distillery effluent on common guppy, *Lesbistes reticulates* and observed remarkable behavioural changes with varying effluent concentration. Kumar and Gopal [18] reported hematological alterations in fresh water catfish, *Channa punctatus*, exposed to distillery effluents. Saxena and Chauhan [19] investigated the influence of distillery effluent on oxygen consumption in fresh water fish, *Labeo rohita* and observed that the presence of inorganic and organic salts in the effluent interfered with the respiration in the fish. The coagulation of gill mucous decreased dissolved oxygen consumption causing asphyxiation. Matkar and Gangotri [20] observed concentration dependent toxicity of distillery effluent on the fresh water crab, *Barythephusa guerini*. Impact of distillery effluent on carbohydrate metabolism of *Cyprinus carpio*, a freshwater fish was studied by Ramakritinan et al. [21]. Stress due to distillery effluent caused defunct respiratory processes in the fish resulting in anaerobiosis at organ level during sublethal intoxication.

Disposal of distillery spent wash on land is equally hazardous to the vegetation. It is reported to reduce soil alkalinity and manganese availability, thus inhibiting seed germination [15]. Kannan and Upreti [22] reported highly toxic effects of raw distillery effluent on the growth and germination of *Vigna radiata* seeds even at low concentration of 5% (v/v). Leaching of protein and carbohydrates from the seeds as well as decrease in activities of important enzymes like alkaline phosphatase and ATPase was also observed.

Table 1
Characteristics of untreated and anaerobically treated distillery effluent [13,14]

Parameters	Values of distillery effluent	Values of anaerobically treated effluent
pH	3.0–4.5	7.5–8
BOD_5 ($mg\,L^{-1}$)	50,000–60,000	8000–10,000
COD ($mg\,L^{-1}$)	110,000–190,000	45,000–52,000
Total solid (TS) ($mg\,L^{-1}$)	110,000–190,000	70,000–75,000
Total volatile solid (TVS) ($mg\,L^{-1}$)	80,000–120,000	68,000–70,000
Total suspended solid (TSS) ($mg\,L^{-1}$)	13,000–15,000	38,000–42,000
Total dissolved solids (TDS) ($mg\,L^{-1}$)	90,000–150,000	30,000–32,000
Chlorides ($mg\,L^{-1}$)	8000–8500	7000–9000
Phenols ($mg\,L^{-1}$)	8000–10,000	7000–8000
Sulphate ($mg\,L^{-1}$)	7500–9000	3000–5000
Phosphate ($mg\,L^{-1}$)	2500–2700	1500–1700
Total nitrogen ($mg\,L^{-1}$)	5000–7000	4000–4200

Application of distillery effluent to soil without proper monitoring, perilously affects the groundwater quality by altering its physicochemical properties such as color, pH, electrical conductivity (EC), etc. due to leaching down of the organic and inorganic ions [23]. In a study carried out by Dhembare and Amin [24], indices indicating soil quality like Sodium Absorption Ratio (SAR), Soluble Sodium Percentage (SSP) and Kelly's ratio were reported to be adversely affected in the soil amended with distillery effluent. Constant disposal/irrigation of the soil with the effluent led to deleterious effect on the soil properties. Soil microorganisms are an essential component of the soil ecosystem and are involved in regulating the various processes of nutrient recycling in soil. Any type of interference with their activity may affect soil productivity as they are the indices of soil fertility. Juwarkar and Dutta [25] evaluated the impact of application of distillery effluent on soil microflora. Irrigation with raw distillery effluent resulted in low overall bacterial and actinomycetes count. However, population of fungi increased. Nitrogen fixing bacteria *Rhizobium* and *Azotobacter* also reduced considerably. Anaerobically treated effluent also showed similar results but not as much as that of the raw effluent.

3. Treatment technologies for distillery spent wash

A number of technologies have been explored for reducing the pollution load of distillery effluent. Biological treatment of distillery spent wash is either aerobic or anaerobic but in most cases a combination of both is used. A typical COD/BOD ratio of 1.8–1.9 indicates the suitability of the effluent for biological treatment [10]. Aerobic treatment of wastes with high organic load such as molasses is associated with operational difficulties of sludge bulking, inability of the system to treat high BOD or COD loads economically, relatively high biomass production and high operational cost in terms of energy requirements [26]. More over a BOD:N:P ratio of 100:2.4:0.3 suggests that anaerobic treatment methods at the primary stage will be more effective than aerobic treatment methods for reducing the pollution potential of distillery effluent. Anaerobic treatment of distillery effluent is an accepted practice and various high rate reactor designs have been tried at pilot and full scale operations [27]. Aerobic treatment of anaerobically treated effluent using different microbes has also been explored. Various physicochemical methods such as adsorption, coagulation–flocculation, and oxidation processes like Fenton's oxidation, ozonation, electrochemical oxidation using various electrodes and electrolytes, nanofiltration, reverse osmosis, ultrasound and different combinations of these methods have also been practiced for the treatment of distillery effluent. These processes are employed generally after the primary anaerobic treatment in order to further reduce the COD and color. Majority of these methods decolorize the effluent by either concentrating the color into the sludge or by breaking down the colored molecules. These treatment technologies are discussed in detail in the following section.

3.1. Anaerobic systems

Anaerobic digestion is viewed as a complex ecosystem in which physiologically diverse groups of microorganisms operate and interact with each other in a symbiotic, synergistic, competitive and antagonistic association. In the process methane and carbon dioxide are generated. The anaerobic microbial food chain consists of mainly three functionally different groups of organism, namely hydrolytic fermentative, syntrophic acetogenic and methanogenic bacteria [28].

Methanogens possess very limited metabolic repertoire, using only acetate or C_1 compounds (H_2 and CO_2, formate, methanol, methylamines or CO), with methane being end product of the reaction. *Methanosarcina* sp. and *Methanosaeta* sp. belonging to the methanogenic genera produce methane by the aceticlastic reaction. Fast growing *Methanosarcina* sp. is predominant in high rate, shorter retention digesters where in acetate concentration is higher. *Methanosaeta* sp. is predominant in low rate slow turn over digesters. Both carbon dioxide reducing and aceticlastic methanogens play an important role in maintaining the stability of the digester. The failure in an anaerobic digester can occur if carbon dioxide reducing methanogens fail to keep pace with hydrogen production [29].

Waste water treatment using anaerobic process is a very promising re-emerging technology which presents interesting advantages as compared to classical aerobic treatment. It has high capacity of degrading concentrated and resilient substances. It produces very little sludge, requires less energy and can become profitable by cogeneration of useful biogas [30]. However, these processes have been sensitive to organic shock loadings, low pH and show slow growth rate of anaerobic microbes resulting in longer hydraulic retention times (HRT). This often results in poor performance of conventional mixed reactors. In order to solve these problems, several high rate configurations have been developed for treating soluble wastewater at relatively shorter HRTs [31].

Realizing the importance of minimum cell residence time (MCRT) as a process control factor, a number of anaerobic processes are available today depending on the way microbial biomass is retained in the reactor. Attempts to overcome the disadvantages of treating different industrial wastes have led to development of various kinds of anaerobic processes [32].

3.1.1. Single-phasic and biphasic anaerobic systems

Anaerobic systems can be operated as single-phase or two-phase systems. Single-phase systems involve only one reactor for the microorganisms to digest the organic matter, whereas two-phase systems separate the acidogenic and methanogenic

organisms into two separate reactors. A biphasic system is capable of optimizing the fermentation steps of each stage in separate fermenters. As a result the overall process efficiency and kinetics are higher than those of conventional single stage processes in which all primary and secondary organisms and associated fermentations are conducted under the same identical environmental conditions. In the primary phase of the fermentation, the end products are formate, acetate, lactate, ethanol, carbon dioxide, hydrogen and C_3 and higher volatile fatty acids. It is basically acid fermentation phase. The secondary phase constitutes acetotrophic methane fermentation where the end products are methane and carbon dioxide [33]. Biomethanation using biphasic system is most appropriate treatment method for high strength waste water because of its multiple advantages viz., possibility of maintaining optimal conditions for buffering of imbalances between organic acid production and consumption, stable performance and higher methane concentration in the biogas produced [34].

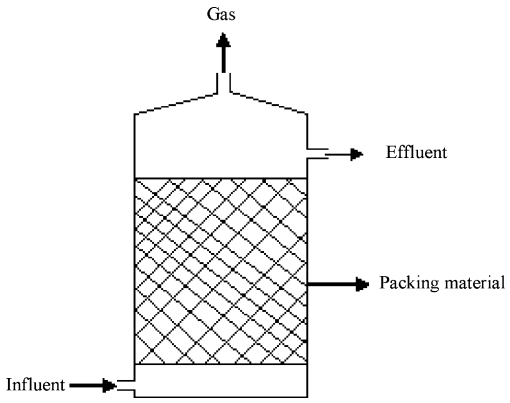

Fig. 1. Schematic diagram of anaerobic fixed film reactor.

3.1.2. Anaerobic lagooning

Anaerobic lagoons are the simplest choice for anaerobic treatment of distillery waste. Rao [35] carried out the pioneering research work in the field of distillery waste management by studying the application of anaerobic lagoon treatment in two pilot-scale lagoons in series, with overall BOD removal ranging from 82 to 92%. However, the lagoon systems are seldom operational, souring being a frequent phenomenon. The ground water contamination cannot be prevented as these lagoons are generally unlined. They require vast area to treat large volumes of the wastes and also lead to odor nuisance [7,10].

3.1.3. Conventional anaerobic systems

The conventional digesters such as continuous stirred tank reactors (CSTR) are the simplest form of closed reactors with provision of gas collection. Treatment of distillery effluent in CSTR has been reported in single as well as biphasic operations, resulting in 80–90% COD reduction within a period of 10–15 days [8]. The HRT in CSTR-type reactor is determined by the specific growth rate of the slowest growing microorganism in the system. This generally means that very high HRT values are required to achieve an acceptable level of degradation. The high HRT values make the CSTR concept less feasible and unattractive for treatment of the wastewaters [36].

3.2. High rate anaerobic reactors

3.2.1. Anaerobic fixed film reactors

In fixed film reactors, the reactor has a biofilm support structure (media) for the biomass attachment. Fig. 1 modified from Kansal et al. [37] shows the schematic representation of an anaerobic fixed film reactor. Fixed film reactor offers the advantages of simplicity of construction, elimination of mechanical mixing, better stability even at higher loading rates and capability to withstand toxic shock loads. The reactors can recover very quickly after a period of starvation [38]. Amongst numerous anaerobic reactors developed for biomethanation, anaerobic fixed film reactors (AFFR) have emerged as the most popular one compared to other reactors due to availability of large biomass in the reactor [39]. The colonization process proceeds in three consecutive phases: lag phase (primary cellular attachment), biofilm production phase (bacterial accumulation with production of biopolymer matrix) and steady state establishment phase (establishment of a mature biofilm) [40]. The nature of the media used for biofilm attachment has a significant effect on reactor performance. A wide variety of materials like glass bead, red drain clay, sand and a number of different plastics and porous materials such as needle punched polyesters, polyureathane foam and sintered glass [41], waste tyre rubber [42], poly(acrylonitrile–acrylamide) [43], corrugated plastic [44], etc., have been used as non-porous support media at laboratory as well as pilot-scale.

Jhung and Choi [45] performed a comparative study of UASB and anaerobic fixed film reactors for treatment of molasses waste. The fixed film reactor was fabricated with a total volume of 5.4 L, filled with Kock plastic media having a porosity of 93–95%, a diameter of 1.6 cm and a specific area of 345 $m^2\,m^{-3}$ and the total volume of the UASB reactor was 4.4 L. The fixed film reactor was found to be more efficient than the UASB reactor as it could be operated at higher OLR (19 kg COD $m^{-3}\,d^{-1}$) than UASB (12.5 kg COD $m^{-3}\,d^{-1}$) and brought about higher COD removal efficiencies at OLR of 10 kg COD $m^{-3}\,d^{-1}$. The better performance of the fixed film reactor was attributed to its ability to retain higher biomass even at higher OLR. Seth et al. [34] carried out comparative studies on the performance of two different support material, namely granular activated carbon (GAC) and clay brick granules (CBG) on biomethanation of distillery spent wash in a biphasic fixed film reactor. The maximum OLR achieved with GAC was 21.3 kg COD $m^{-3}\,d^{-1}$, corresponding to a HRT of 4 days with COD and total volatile fatty acids (TVA) reductions of 67% and 82%, respectively, where as OLR achieved with CBG was 22 kg COD $m^{-3}\,d^{-1}$, corresponding to a HRT of 3 days with COD and TVA reductions of 71.8% and 88.5%, respectively. The enhanced performance of CBG over GAC as support material was attributed to the better support characteristics of the former as confirmed by scanning electron microscopy analysis.

Thermophilic stability of the fixed film reactors was investigated by Perez et al. [41] using anaerobic fixed film reactor packed with porous sintered-glass support. This carrier termed as SIRAN, was produced by sintering of a mixture of glass and salt powder. The resulting sponge had a well defined pore size distribution (double pore structure) which resulted in 80% COD reduction at a COD loading rate 3.81 kg COD $m^{-3}\,d^{-1}$ in 75 days. This study revealed that under thermophilic anaerobic conditions the support material enabled faster attachment of the microorganisms resulting in shorter start up and stable operation. In another study, Perez-Garcia et al. [44] studied the influent pH conditions in fixed film reactors for anaerobic thermophilic treatment of wine distillery wastewaters. The results obtained showed that the pH of the influent influenced the performance of the biodegradation process and the depurative efficiency was higher with alkaline influent. The operation with acidic influent allowed the reactor to operate at OLR around 5.6 kg COD $m^{-3}\,d^{-1}$ (HRT: 1.5 days), maintaining total Chemical Oxygen Demand removals (CODr) of 77.2%; the operation with alkaline influent allowed total CODr of 76.8% working at OLR around 10.5 kg COD $m^{-3}\,d^{-1}$. The greatest efficiency of

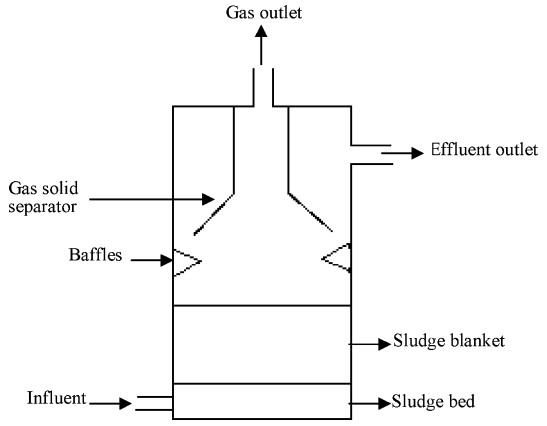

Fig. 2. Schematic diagram of anaerobic UASB reactor.

substrate removal was 87.5% for OLR 3.2 kg COD m^{-3} d^{-1} and HRT of 4 days operating with alkaline influent. Therefore, the operation with alkaline influent implicates higher levels of purifying efficiency for similar organic load rate. Acharya et al. [14] performed a comparative study of low cost packing materials for the treatment of distillery spent wash using anaerobic fixed film reactors. Coconut coir was found to be the best supporting material, as the system supported the treatment at very high organic loading rate of 31 kg COD m^{-3} d^{-1} with 50% COD reduction. Charcoal and Nylon fibers were other packing materials used in the study. Charcoal was able to retain the active biomass at the OLR of 15.5 kg COD m^{-3} d^{-1} resulting in more than 60% COD reduction whereas nylon fibers failed to support the biofilm development even at higher HRT and lower OLR.

3.2.2. Upflow anaerobic sludge blanket (UASB) reactors

In the recent years, the UASB process has been successfully used for the treatment of various types of wastewaters [46]. UASB reactor systems belong to the category of high rate anaerobic wastewater treatment and hence it is one of the most popular and extensively used reactor designs for treatment of distillery wastewaters globally. The success of UASB depends on the formation of active and settleable granules [47]. These granules consist of aggregation of anaerobic bacteria, self immobilized into compact forms. This enhances the settelability of biomass and leads to an effective retention of bacteria in the reactor [48]. Particularly attractive features of the UASB reactor design includes its independence from mechanical mixing of digester contents, recycling of sludge biomass [49] and ability to cope up with perturbances caused by high loading rates and temperature fluctuations [50]. Fig. 2 modified from Kansal et al. [37] shows the schematic representation of an upflow anaerobic sludge blanket (UASB) reactor. The UASB technology is well suited for high strength distillery wastewaters only when the process has been successfully started up and is in stable operation. To achieve successful startups, the reactors must be operated at a low loading rate of 4–8 kg COD m^{-3} d^{-1} and the COD removal efficiency must be monitored carefully. The loading rate can be increased, when the COD removal efficiencies are above 90% [51]. Malt whisky distillery potale, a liquid waste product from the malt whisky industry, treated in a laboratory scale UASB reactor showed the effect of dilution and pH control in attaining a high COD reduction [52]. There is normally a rise in the pH due to ammonia production during the process of digestion. The maximum loading rate for a stable operation was 15 kg COD m^{-3} d^{-1} at a retention time of 2.1 days. Florencio et al. [53] investigated the environmental factors that are of importance in the predominance of methylotrophic methanogens over acetogens in a natural mixed culture during anaerobic treatment in UASB reactors. An increased growth rate of the methanogens at higher temperatures makes the thermophilic anaerobic digestion process a suitable alternative to mesophilic digestion [38]. Harada et al. [54] investigated the feasibility of UASB reactors at thermophilic temperatures. A 140 L UASB reactor was studied for a period of 430 days. The organic loading rate up to 28 kg COD m^{-3} d^{-1} was applied by reducing HRT at a fixed influent concentration of 10 kg COD m^{-3} d^{-1}. The removal of COD was about 67% while BOD removal was more significant (more than 80%). Successful operation of the UASB reactors for treating distillery waste at psychrophilic temperatures (4–10 °C) was studied by operating one and two-stage UASB reactors. The organic loading rate varied from 4.7 to 1.3 g COD at HRT of 6–7 days for one-stage reactor and 2 days for the two-stage reactor. The average total COD removal for vinasses waste waters was 60% in the one-stage reactor and 70% in the two-stage reactor. In situ determinations of kinetic sludge characteristics (apparent Vm and Km) revealed the existence of substantial mass transfer limitations for the soluble substrates inside the reactor sludge bed. Therefore, application of higher recycle ratios is essential for enhancement of UASB pretreatment under psychrophilic conditions [55]. The conventional UASB reactors concept showed severe limitations mainly owing to problems related to mass transfer resistance or the appearance of concentration gradients inside the systems [56]. Some of the other disadvantages of the process are slow primary startup requiring several weeks, difficulty in controlling granulation process which depends upon a large number of parameters. As the organic loading increases, the process needs to be properly monitored to maintain required alkalinity to balance excessive acid accumulation [57]. In last decades, the system specific parameters of UASB reactors have been modified to increase the loading potentials and/or to widen the applicability of anaerobic reactor systems for various types of waste waters [56]. By making use of the high settleability of the methanogenic sludge granules (40–60 mh^{-1}), Expanded Granuler Sludge Bed (EGSB) systems have been developed. These are operated at up flow velocities exceeding 8 mh^{-1}, which is brought about by an increased height diameter ratio and external circulation pump. In contrast to the conventional UASB reactors the EGSB systems are not equipped with an internal settler but with an advanced gas–liquid–solid separation device [56]. As a result of the excellent contact between wastewater and the sludge these systems can handle higher organic loading rates. As compared to conventional UASB systems, they are less sensitive to negative effects of suspended solids present in wastewater [58].

Anaerobic treatment of low-strength brewery wastewater, with influent total chemical oxygen demand (CODin) concentrations ranging from 550 to 825 mg L^{-1}, was investigated in a pilot-scale 225.5 L expanded granular sludge bed (EGSB) reactor. At 20 °C, COD removal efficiencies exceeding 80% were obtained at an OLR up to 12.6 kg COD m^{-3} d^{-1}, with CODin between 630 and 715 mg L^{-1}. The values of HRT and liquid upflow velocity applied were 2.1–1.2 h, and 4.4–7.2 mh^{-1}, respectively. The acidified fraction of the CODin was above 90%, but sludge washout was not significant. These results indicate that the potentials of EGSB reactors can be further explored for the anaerobic treatment of low-strength brewery wastewater, even at lower temperatures [59].

A significant improvement in UASB system was achieved by modifying the reactor and operating these modules in series. Akunna and Clark [48] proposed a hybrid reactor, which was a combination of UASB and an anaerobic baffled reactor for treatment of high strength wastewaters, referred to as Granular Bed Anaerobic Baffled Reactor (GRABBR). Up to 80% of COD and 90% of BOD removal was observed for organic loading rate of 4.75 kg COD m^{-3} d^{-1}. Biogas production increased with increasing loading rates from 10

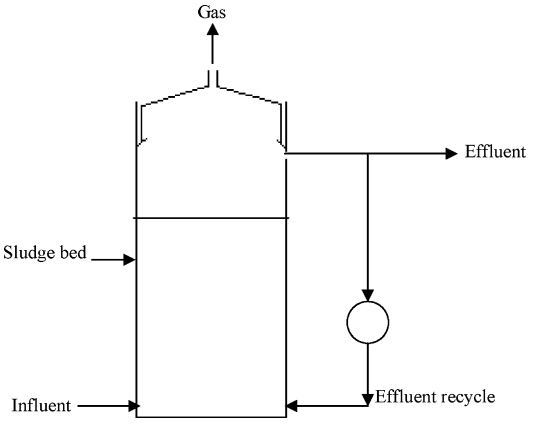

Fig. 3. Schematic diagram of anaerobic fluidized bed reactor.

to 22 L d^{-1} for loading rates 0.99 and 4.75 kg COD m^{-3} d^{-1}, with 60–70% methane content. The effectiveness of the reactor stemmed from the process stability created by the phase separation provided in the reactor configuration. The system also showed very high solids retention with effluent suspended solids concentration of about 80 mg L^{-1} for all organic and hydraulic conditions. This was attributed to the occurrence of granular methanogens in the downstream of zone occupied by non-granular acidogens.

Upon realizing the potential advantages of biphasic biomethanation, Uzal et al. [60] investigated the anaerobic treatment of whisky distillery waste in two-stage UASB reactors and concluded that the system worked efficiently even at OLRs as high as 39 kg COD m^{-3} d^{-1} resulting in 95–96% COD reduction.

3.2.3. Anaerobic fluidized bed reactors

In the anaerobic fluidized bed reactor (AFB) the medium for bacterial attachment and growth is kept in the fluid state by drag forces exerted by the up flowing wastewater. The media used are small particle size sand, activated carbon, etc. In the fluidized state, each medium provides a large surface area for biofilm formation and growth. It enables the attainment of high reactor biomass hold-up and promotes system efficiency and stability. Fluidized bed technology is an effective anaerobic technology for treatment of high strength waste waters as it favors the transport of microbial cells from the bulk to the surface and thus enhances the contact between the microorganisms and the substrate [61]. Fig. 3 modified from Kansal et al. [37] shows the schematic representation of an anaerobic fluidized bed reactor. Kida et al. [62] studied the biological treatment of Shochu distillery wastewater using an anaerobic fluidized bed reactor. By the addition of nickel, cobalt and diluting the waste, maximum loading rate of 22 kg TOC m^{-3} d^{-1} could be achieved. This resulted in 70% TOC (total organic carbon) reduction. Ability of anaerobic fluidized bed reactor to treat high strength wastewaters like distillery waste under thermophilic temperatures was studied by Perez et al. [41]. It was confirmed that AFB systems can achieve >82.5% COD reduction at a COD loading rate of 32.3 kg COD m^{-3} d^{-1} corresponding to HRT of 0.46 day. The greatest efficiency of substrate removal was 97% for an organic loading rate of 5.9 kg COD m^{-3} d^{-1} and HRT of 2.5 days. The food-to-microorganism (F:M) ratio can be used as a parameter for performance evaluation of AFB. For the effluent, excellent COD reduction and methane production were achievable at the F:M ratio of 0.55 kg COD kg^{-1} VSatt d^{-1}. At this F:M ratio, more than 80% of feed COD was removed and 9 m^3 m^{-3} d^{-1} of methane was produced. Perez-Garcia et al. [44] compared the performance of two high rate technologies viz. upflow anaerobic fixed film reactor and anaerobic fluidized bed reactor. They concluded that the fluidized bed reactor, operating on open pore sintered-glass media, gives total COD removal of 96% at OLR_0 of 5.88 kg COD m^{-3} d^{-1}.

Fundamentally, the anaerobic fluidized bed technology is more effective than the upflow anaerobic fixed film technology, as this favors the transport of microbial cells from the bulk to the surface and thus enhancing the contact between the microorganism–substrate phases. In principle, application of fluidized bed reactor overcomes mass transfer limitations. However, these systems are difficult to manage because of problems of biofilm stability due to shear stresses or to bed segregation from the inert support material. Moreover, to obtain complete fluidization, the energy requirement of fluidized bed reactors is relatively very high [56].

3.2.4. Anaerobic batch reactors

Treatment of distillery waste using batch reactors has not been widely attempted. Reactor potential, operational feasibility and scale up of such reactors needs to be explored. Treatment of winery wastewater was investigated using an anaerobic sequencing batch reactor (ASBR). The reactor was operated at an OLR of 8.6 kg COD m^{-3} d^{-1} with soluble COD removal efficiency greater than 98%, HRT of 2.2 days [63]. Banerjee and Biswas [64] designed a semi continuous batch digester to investigate biomethanation of distillery waste in mesophilic and thermophilic range of temperatures. The study revealed that there is an enormous effect of digestion temperature and substrate concentration in terms of BOD and COD loading on the yield of biogas as well as its methane content. Maximum BOD reduction (86.01%), total gas production and methane production (73.23%) occurred at a BOD loading rate of 2.74 kg m^{-3} at 50 °C digestion temperature.

3.2.5. Novel anaerobic reactors

Innovative research into bioreactor designs for treatment of high strength waste like distillery effluent, has led to the development of novel bioreactors. To overcome the difficulties of substrate feeding during the start up and to prevent excessive accumulation of volatile fatty acids, Uyanik [65] developed the Split Fed Anaerobic Baffled Reactor (SFABR). The potential advantage of the SFABR over the normally fed ABR includes reduction in the severity of conditions (toxicity) in the initial compartments. Split feeding prompted balanced gas production between compartments and improved mixing pattern in the reactor. Distillery effluent was fed into the reactor at an OLR of 10.5 kg COD m^{-3} d^{-1} and after 70 days of operation, 90% COD reduction was observed. Arnaiz et al. [66] designed an inverted turbulent bed reactor for treatment of wine distillery waste using pre-colonized bioparticles. The reactor was a modification of inverse fluidized bed showing advantages in terms of higher sludge recovery, higher liquid recycling, reduced clogging problems and lower energy requirements due to low fluidization rates. The maximum OLR achieved by the reactor was 28.2 kg COD m^{-3} d^{-1} corresponding to an HRT of 11.2 h resulting in about 92% COD reduction. Kumar et al. [67] carried out the biomethanation of distillery spent wash in an anaerobic hybrid reactor (combining sludge blanket and filter) in a continuous mode. The study demonstrated that at optimum HRT of 5 days and at an OLR of 8.7 kg COD m^{-3} d^{-1}, the COD removal efficiency of the reactor was 79% and concluded that anaerobic hybrid reactor could be successfully employed for treatment of distillery spent wash. Fig. 4 modified from Kansal et al. [37] shows the schematic representation of an anaerobic hybrid reactor. Sowmeyan and Swaminathan [68] recently designed an inverse anaerobic fluidized bed reactor containing perilite as the carrier material and found it to be a better choice among the different anaerobic methods available for treatment of distillery effluent. The system when operated at

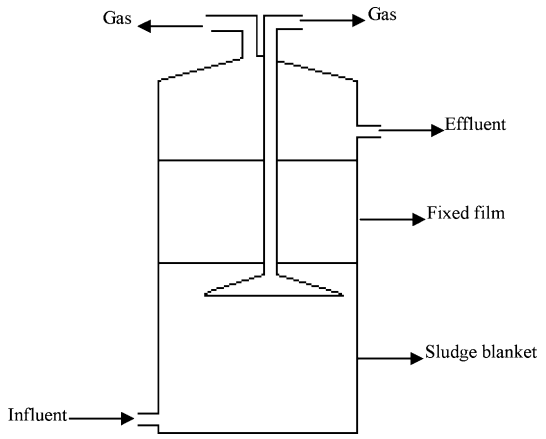

Fig. 4. Schematic diagram of anaerobic hybrid reactor.

35 kg COD m^{-3} d^{-1} and HRT of 0.19 day resulted in 84% COD reduction.

3.3. Aerobic systems

Anaerobically treated distillery spent wash still contains high concentrations of organic pollutants and as such cannot be discharged directly. The partially treated spent wash has high BOD, COD and suspended solids. It has high C:N ratio (>20). It can reduce the availability of important mineral nutrients by trapping them into immobile organic forms, and may produce phytotoxic substances during decomposition. It is thus unsuitable for irrigation. Stringent regulations on discharge of colored effluent impede direct discharge of anaerobically treated effluent [11]. Colorants encountered in sugarcane processing are normally biopolymeric colloidal materials that are negatively charged. All colorants, except caramel contain phenolics groups which contribute to the formation of

Table 2
White rot fungi employed for treatment of distillery effluent

Culture	Treatment	COD removal	Color removal	Enzymes	Reference
Coriolus sp. No. 20	Synthetic melanoidin solution was decolorized by the fungus	NR	80%	Sorbose oxidase	[79]
Phanerochaete chrysosporium	Free cells as well as Ca alginate immobilized cells decolorized the distillery effluent.	NR	85% (free) 59% (immobilized)	NR	[80]
Trametes versicolor	Anaerobically treated distillery effluent supplemented with sucrose and inorganic N sources was decolorize by the culture in shake flask studies	75%	80%	NR	[81]
Phanerochaete chrysosporium	Both the cultures decolorized to reduced the COD of effluent in presence of (3–5%) glucose and 0.1% yeast extract	73%	53.5%	NR	[71]
Coriolus versicolor		70%	71.5%	NR	
Coriolus hirsutus	Synthetic as well as wastewater melanoidin was decolorized by the fungus in a medium containing glucose and peptone.	NR	80%	MiP and MnP and presence of extracellular H_2O_2	[82,83]
Coriolus hirsutus IF044917	The fungal culture was immobilized on PUF and used for decolorization of melanoidins present in heat treated liquor	NR	45%	NR	[84]
Flavodon flavus	Distillery effluent was decolorized using this marine basidiomycetes in presence of 5% glucose.	NR	80%	Glucose oxidase accompanied with hydrogen peroxide	[85,86]
Coriolus versicolor	The cultures were incubated along with cotton stalks in vinasses, media in static condition. No synthetic carbon or nitrogen sources were used.	49	63	NR	[87]
Funalia trogii		62	57		
Phanerochaete chrysosporium		57	37		
Pluereotus pulmonaris		34	43		
Phanerochaete chrysosporium 1557	Effect of Veratryl alcohol and Mn (II) on decolorization of distillery effluent was studied.	NR	75%	LiP and MnP	[88]
Phanerochaete chrysosporium ATCC 24725	The fungus was immobilized on different support materials such as PUF and scouring wet and the decolorization was carried out in a RBC	48%	55%	NR	[89]
P. chrysosporium NCIM 1073	The cultures were employed to study the decolorization of molasses in medium containing 2% w/w glucose in static as well as submerged conditions.	Nil	Nil	NR	[90]
NCIM 1106		NR	82%	LiP and MnP	
NCIM 1197		NR	76%	LiP and MnP	
Marine basidiomycetes NIOCC	Experiments were carried out with 10% (v/v) diluted effluent	NR	100%	Laccase and exoploysaccharide produced by the fungus	[91]

NR: Not reported.

colorants. IR spectra of alkaline degradative products indicate the presence of ionisable, high molecular weight amino acids. It has been suggested that most of the phenolic colorants are derived from benzoic and cinnamic acid that are precursors of flavanoids, the yellow plant pigments responsible for color formation. The phenolics acids which form colored complexes with iron or get oxidized to polymeric colorants are *o*-hydroxy or *o*-dihydroxy acids [69]. During heat treatment, the Maillard reaction takes place resulting in formation of melanoidins, one of the final products of the Maillard reaction [2,10,13,15].

Aerobic treatment of anaerobically treated distillery spent wash has been attempted for the decolorization of the major colorant, melanoidins and for further reduction of the COD and BOD. A large number of microorganisms such as bacteria (pure and mixed culture), cyanobacteria, yeast, fungi, etc. have been isolated in recent years that are capable of degrading melanoidins and thus decolorizing the waste.

3.3.1. Fungal systems

Fungi are recognized by their superior aptitude to produce a large variety of extracellular proteins, organic acids and other metabolites and for their capacity to adapt to severe environmental constraints [70]. Increasing attention has been directed towards utilizing microbial activity for decolorization of molasses spent wash. Several reports have indicated that some fungi in particular have such a potential [71]. One of the most studied fungus having ability to degrade and decolorize distillery effluent is *Aspergillus* sps. *Aspergillus fumigatus* G-2-6, *Aspergillus niger*, *A. niveus*, *A. fumigatus* $U_{B2}60$ brought about an average of 69–75% decolorization along with 70–90% COD reduction [72,73,26,74–76]. Treatment of distillery spent wash with ascomycetes group of fungi such as *Penicillium* spp., *Penicillium decumbens*, *Penicillium lignorum* resulted in about 50% reduction in color and COD, and 70% phenol removal [26]. Sirianuntapiboon et al. [77] reported an absorption mechanism for decolorization of melanoidins by *Rhizoctonia* sps. D-90. The pigments were accumulated in the cytoplasm and around the cell membrane as melanoidin complex, which was then gradually decolorized by intracellular enzymes.

White rot fungi is another group of widely exploited microorganism in distillery effluent bioremediation. White rot fungi produce various isoforms of extracellular oxidases including laccases, manganese peroxidases and lignin peroxidase, which are involved in the degradation of lignin in their natural lignocellulosic substrate. This ligninolytic system of white rot fungi is directly involved in the degradation of various xenobiotic compounds and dyes [78]. Table 2 gives details about different white rot fungi employed in decolorization of distillery effluent and the role of different enzymes in the process.

Recently, Pant and Adholeya [92] isolated three fungal cultures and identified them by molecular methods as *Penicillium pinophilum* TERI DB1, *Alternaria gaisen* TERI DB6 and *Pleurotus florida* EM 1303. These cultures were found to produce ligninolytic enzymes and decolorized the effluent up to 50%, 47% and 86%, respectively.

3.3.2. Bacterial systems

Different bacterial cultures capable of both bioremediation and decolorization of anaerobically treated distillery spent wash have been isolated. Kumar and Viswanathan [93] isolated bacterial strains from sewage and these strains were able to reduce the COD of the distillery effluent by 80% after 4–5 days. Kumar et al. [15] isolated a facultative anaerobic pure bacterial culture L-2, a gram positive non-motile rod belonging to Genus Lactobacilli. The culture was able to decolorize the effluent by 31% and remove 57% COD of 12.5% diluted waste water supplemented with 10 g L^{-1} glucose in 7 days. Nakajima et al. [94] isolated a *Bacillus* sp. which decolorized molasses waste water up to 35.5% within 20 days at 55 °C (thermophilic conditions) under anaerobic conditions. The molecular weight distribution as determination by gel permeation chromatography revealed that there was decrease in color contributing small molecules as well as large molecules.

Some researchers carried out melanoidin decolorization by using immobilized whole cells. Ohmomo et al. [95] used calcium alginate immobilized cells of *Lactobacillus hilgardi* to decolorize melanoidin solution which resulted in 40% decolorization. Decolorization of molasses wastewater by immobilized cells of *Pseudomonas fluorescence* on porous cellulose carrier was attempted achieving 76% decolorization in 24 h at 30 °C. Cellulose carrier coated with collagen was found to be most efficient carrier, which could be reused with 50% decolorization activity retained until the seventh day [96]. Jain et al. [97] isolated three bacterial cultures from the activated sludge of a distillery waste water plant identified as *Xanthomonas fragairae*, *B. megaterium* and *B. cereus* which were found to remove COD and color from the distillery effluent in the range of 55–68% and 38–58%, respectively. Two bacterial strains *Pseudomonas putida* U and *Aeromonas* strain Ema were used to bioremediate anaerobically treated distillery spent wash in a two-stage bioreactor. In the first stage, *P. putida* reduced the COD and color by 44.4% and 60%, respectively. The *Aeromonas* strain Ema, in the second stage, reduced the COD by 44%. Algal bioassay was used to evaluate the quality of the spent wash before and after treatment. The spent wash was eutrophic before the experimental treatment, but, after treatment, it showed poor algal growth [98]. In another study, Ghosh et al. [99] isolated, identified and elucidated the phylogenetic relationship of a number of bacterial strains capable of using recalcitrant compounds of molasses spent wash as sole carbon source and thus reducing the COD of the waste. Six strains, namely *Pseudomonas*, *Enterobacter*, *Aeromonas*, *Stenotrophomonas*, *Acienatobacter* and *Klebsiella* brought about 44% COD reduction of the distillery effluent. However, no decolorization was observed. Sirianuntapiboon et al. [100] isolated an acetogenic strain from vegetable and juice samples which decolorized the molasses pigment medium and anaerobically treated distillery effluent to 73–76% within 5 days when supplemented with glucose and nitrogen sources. In replacement culture system involving six replacements, the strain showed constant decolorization and decrease in BOD and COD values of 58.5–82.2% and 35.5–71.2%, respectively.

Sangave and Pandit [101] proposed a combined treatment technique consisting of enzymatic hydrolysis by cellulases followed by aerobic oxidation with a gram positive culture ASN 6. The rate of aerobic oxidation was enhanced by 2.3-fold for pretreated sample as compared to untreated sample. In another study, Sangave and Pandit [102] used a combination of irradiation with ultrasound and hydrolysis with cellulase prior to aerobic oxidation of the effluent with the culture ASN 6. This resulted in a 4-fold increase in the initial oxidation rate over the untreated batch of effluent.

Mixed culture studies have been carried out by several researchers for degradation of different effluents such as textile effluents. As the catabolic activities of microorganisms in a mixed consortium complement each other, obviously the syntrophic interactions present in mixed communities lead to complete mineralization of the effluent [103]. The decolorization of four synthetic melanoidins (i.e., GGA, GAA, SGA, and SAA) by three *Bacillus* isolates, namely *Bacillus thuringiensis* (MTCC 4714), *Bacillus brevis* (MTCC 4716) and *Bacillus* sp. (MTCC 6506) was studied by Kumar and Chandra [104]. A mixed culture comprising of these three strains brought about significant reduction in the values of physicochemical parameters along with the decolorization of all four types of melanoidins (10%, v/v). The medium that contained glucose as a sole carbon source showed 15% more decolorization than

Table 3
Summary of various physicochemical treatments used for the treatment of distillery spent wash and their efficiency

Treatment	%COD removal	%Color removal	Reference
Adsorption			
Chitosan, a biopolymer was used as anion exchanger	99	98	[113]
Chemically modified bagasse			
DEAE bagasse	40	51	[69]
CHPTAC bagasse	25	50	
Activated carbon prepared from agro industrial waste			
Phosphoric acid carbonized bagasse was used	23	50	[114]
Commercially available activated carbon			
AC (ME)	76	93	
AC (LB)	88	95	
Coagulation–flocculation			
Flocculation of synthetic melanoidins was carried out by various inorganic ions			
Polyferric hydroxysulphate (PFS)	NR	95	
Ferric chloride ($FeCl_3$)	NR	96	
Ferric sulphate ($Fe_2(SO_4)_3$)	NR	95	
Aluminium sulphate ($Al_2(SO_4)_3$)	NR	83	[115]
Calcium oxide (CaO)	NR	77	
Calcium chloride ($CaCl_2$)	NR	46	
Different inorganic ions and waste water from Iron pickling and Titanium process industry were used as coagulants. Addition of polyelectrolyte Percol 47 reduced their dosage			
Ferrous sulphate ($FeSO_4$)	78	98	
Ferric sulphate ($Fe_2(SO4)_3$)	77	96	
Alum	64	95	[12]
Iron pickling waste water	86	99	
Titanium processing waste water	67	99	
Iron chloride coagulation	38	47	[116]
Iron chloride	65	69	
Aluminium chloride	61.3	74.4	[117]
Calcium oxide	39.8	80.2	
Ferric chloride ($FeCl_3$)	55	83	[118]
Aluminium chloride ($AlCl_3$)	60	86	
Polyaluminium chloride (PAC)	72	92	
Oxidation processes			
Fenton's oxidation	88	99	[119]
Ozonation	15–25	80	[112]
Electrochemical oxidation			
Graphite electrodes	80.6	95.6	
Lead dioxide coated on titanium	90.8	98.5	[120]
Ruthedium dioxide coated on titanium	92.1	99.5	
Electrocoagulation and electro Fenton	92.6	–	[121]
Membrane technologies			
Reverse osmosis	99.9	–	[122]
Nanofiltration	97.1	100	

NR: Not reported.

that containing both carbon and nitrogen sources. The addition of 1% glucose as a supplementary carbon source was essential for co-metabolism of melanoidin complex. The toxicity of synthetic melanoidin to tubificid worm (*Tubifex tubifex*, Müller), was significantly reduced upon decolorization by the three *Bacillus* sp. Chaturvedi et al. [105] isolated and characterized fifteen culturable rhizosphere bacteria of *Phragmites australis* growing in distillery effluent contaminated sites. These fifteen cultures were *Microbacterium hydrocarbonoxydans*, *Achromobacter xylosoxidans*, *Bacillus subtilis*, *B. megaterium*, *B. anthracis*, *B. licheniformis*, *A. xylosoxidans*, *Achromobacter* sp., *B. thuringiensis*, *B. licheniformis*, *B. subtilis*, *Staphylococcus epidermidis*, *Pseudomonas migulae*, *Alcaligens faecalis*, *B. cereus* which collectively brought about 76% decolorization and 85–86% BOD and COD reduction of the effluent within 30 days. A novel bacterial consortium comprising of *Pseudomonas aeruginosa* PAO1, *Stenotrophomonas maltophila* and *Proteus mirabilis* has been isolated from distillery effluent contaminated sites following enrichment culture by Mohana et al. [13]. This consortium exhibited rapid degradation of the effluent resulting in 67% decolorization and 51% COD reduction within 72 h in presence of very low nutrient medium.

3.3.3. Cyanobacterial/algal systems

Cyanobacteria are considered ideal for treatment of distillery effluent as they, apart from degrading the polymers also oxygenate waterbodies, thus reduce the BOD and COD levels. Kalavathi et al. [106] explored the possibility of using a marine cyanobacterium for decolorization of distillery spent wash and its ability to use melanoidins as carbon and nitrogen source. A marine filamentous, non-heterocystous form *Oscillatoria boryana* BDU 92181 used the recalcitrant biopolymer melanoidin as nitrogen and carbon source leading to decolorization. Indirect evidence through the study of nitrogen assimilating enzymes as well as direct evidence of using ^{14}C radiolabeled synthetic melanoidins confirmed this ability. The organism decolorized pure melanoidin pigment (0.1%, w/v) by about 75% and crude pigment in the distillery effluent (5%, v/v)

by about 60% in 30 days. The mechanism of color removal is postulated to be due to the production of hydrogen peroxide, hydroxyl anions and molecular oxygen, released by the cyanobacterium during photosynthesis. Valderrama et al. [107] studied the feasibility of combining microalgae, *Chlorella vulgaris* and macrophyte *Lemna minuscule* for bioremediation of wastewater from ethanol producing units. This combination resulted in 61% COD reduction and 52% color reduction. First, the microalgal treatment led to removal of organic matter and further treatment with macrophytes removed other organic matter, color and precipitated the microalgae.

3.3.4. Phytoremediation/constructed wetlands

Phytoremediation of effluents is an emerging low cost technique for removal of toxicants including metals from industrial effluents and is still in an experimental stage. Aquatic plants have excellent capacity to reduce the level of toxic metals, BOD and total solids from the wastewaters [108]. Billore et al. [109] carried out the treatment of distillery effluent in a constructed wetland which comprised of four cells. After a pretreatment in the cells one and two the effluent was channeled to cells three and four which contained plants *Typha latipholia* and *Phragmites karka*. This treatment eventually led to 64% COD, 85% BOD, 42% total solids and 79% phosphorus content reduction. An aquatic macrophyte *Potamogeton pectinatus* was found to bioaccumulate metals (Fe, Cu, Zn and Mn) and efficiently cleanup the effluent [110]. Kumar and Chandra [108] successfully treated distillery effluent in a two-stage process involving transformation of recalcitrant coloring components of the effluent by a bacterium *Bacillus thuringienesis* followed by subsequent reduction of remaining load of pollutants by a macrophyte *Spirodela polyrrhiza*. A similar biphasic treatment of the effluent was carried out in a constructed wetland with *B. thuringienesis* and *Typha angustata* by Chandra et al. [111] which resulted in 98–99% BOD, COD and color reduction after 7 days.

3.4. Treatments based on physicochemical methods

After a multistage biological treatment of distillery spent wash, most of the organic load is removed. However, the brown color does not disappear and may even increase due to repolymerization of the colored components, melanoidins [112]. Conventional anaerobic and aerobic treatment can accomplish degradation of the melanoidins up to only about 6–7%. Therefore, it is necessary to study about additional treatments required to decolorize distillery effluent [112]. Melanoidins have been reported to be decolorized by various physiochemical methods which are summarized in Table 3. Majority of these methods remove color by either concentrating the color into sludge or by partial or complete breakdown of the color molecules.

3.4.1. Adsorption

Among the physicochemical treatment methods, adsorption on activated carbon (AC) is widely employed for removal of color and specific organic pollutants. Activated carbon is a well known adsorbent due to its extended surface area, microporus structure, high adsorption capacity and high degree of surface reactivity. Previous studies on decolorization of distillery spent wash include adsorption on commercial as well as indigenously prepared activated carbons [4].

3.4.2. Coagulation and flocculation

Coagulation is the destabilization of colloids by neutralizing the forces that keep them apart. Cationic coagulants provide positive electric charges to reduce the negative charge (zeta potential) of the colloids. As a result, the particles collide to form larger particles (flocs). Flocculation is the action of polymers to form bridges between the flocs, and bind the particles into large agglomerates or clumps. Bridging occurs when segments of the polymer chain adsorb on different particles and help particles aggregate. Generally coagulation seems to be an expensive step taking into account expenses of chemicals and sludge disposal. Thus, there is a need for development of low cost alternatives for post biomethanated effluent [123].

3.4.3. Oxidation processes

Ozone is a powerful oxidant for water and waste water treatment. Once dissolved in water, ozone reacts with a great number of organic compounds in two different ways: by direct oxidation as molecular ozone or by indirect reaction through formation of secondary oxidants like free radical species, in particular the hydroxyl radicals. Both ozone and hydroxyl radicals are strong oxidants and are capable of oxidizing a number of compounds [124].

The Fenton's oxidation technology is based on the production of hydroxyl radicals $^{\bullet}OH$, which have an extremely high oxidation potential. Fenton's reagent, which involves homogeneous reaction and is environmentally acceptable, is a mixture of hydrogen peroxide and iron salts (Fe^{2+} or Fe^{3+}) which produces hydroxyl radicals which ultimately leads to decolorization of the effluent [119].

3.4.4. Other treatments

Pikaev [125] applied radiation technology for treatment of distillery waste. The study involved a combined treatment of electron beam (dose 20 kGy) and coagulation using $Fe_2(SO)_3$ which resulted in a decrease in optical absorption in the *uv* region by 65–70% in the treated effluent. Ultrasound technology was also applied for the treatment of distillery effluent. Studies were carried out to find out the efficacy of the ultrasonic irradiation as a pretreatment step and the results indicated that ultrasound treatment enhanced the biodegradability of the distillery waste water [126]. Chaudhari et al. [127] proposed a novel catalytic thermal pretreatment or catalytic thermolysis (CT) to recover the majority of its energy content with consequent COD and BOD removal. They found that the initial pH (pH_0) had profound impact on the efficiency of thermolysis in COD removal. At 140 °C with 3 kg m^{-3} catalyst loading and pH_0 2 (optimum value), they observed a maximum of 60% COD removal. The CT process resulted in the formation of settleable solid residue and the slurry obtained after the thermolysis exhibited very good filtration characteristics. At 140 °C and pH_0 2, the solid residue had a C:H atomic ratio of 1:1.08 with a heating value of 21.77 MJ kg^{-1}. The residue can be used as a fuel in the combustion furnaces and the ash obtained can be blended with organic manure and used in agriculture/horticulture. Kannan et al. [128] adopted electrocoagulation technique with addition of indigenously prepared areca nut carbon (AAC) for treatment of distillery effluent. This study, for a period of 1 h, resulted in almost colorless effluent with 89.7% BOD and 80% COD removal.

4. Potential applications of distillery spent wash

Distillery effluent after anaerobic treatment still contains considerable plant nutrients in terms of potassium, sulphur, nitrogen and phosphorus. Moreover, it contains large amount of micronutrients like Ca, S, Cu, Mn and Zn. Various researchers have reported that irrigation with distillery effluent increased crop yield, dry matter, leaf area, total chlorophyll, etc. [129–131].

Pathak et al. [129] performed a field study in which soil amended with diluted distillery effluent increased the yield of wheat and rice. Organic carbon and available potassium content of post harvest soils had also increased. Ramana et al. [130] studied the relative efficacy of different distillery effluents (raw, biomethanated and

lagoon sludge) on growth, nitrogen fixation and yield of groundnut. Among the three effluents, biomethanated effluent supported highest seed yield (619 kg ha^{-1}) followed by raw distillery effluent (557 kg ha^{-1}) and lagoon sludge (472 kg ha^{-1}). However, with control (water) the seed yield was only 310 kg ha^{-1}. Parallel study was performed using maize which gave similar results [132]. Orhue et al. [133] also reported the positive effect of distillery effluent on the growth of maize. In another study Ramana et al. [134] assessed the effect of distillery effluent on seed germination in some crops such as cucumber, chilli, onion, bottle gourd and tomato. They concluded that higher effluent concentration inhibited germination and the effect of effluent was also found to be crop specific. In order to overcome the negative effects of long term application of distillery effluent on soil, the effluent was applied to the sodiac soil in combination with bioamendments like farmyard manure, brassica residues and rice husk in a study conducted by Kaushik et al. [135]. This resulted in significant increase of TOC, TKN (total Kjeldhal nitrogen), potassium, phosphorus and soil enzymatic activity and also favored successful germination and improved seedling growth of pearl millet, but with researchers reporting beneficial as well as detrimental effects, the use of distillery effluent in agriculture remains controversial [129].

One of the procedures followed for the disposal of distillery effluent is using it for production of microbial biomass. Conversion of large part of the organic loadings of the distillery effluent to microbial biomass has special relevance to countries where supplementation of food and feeds is an urgent necessity. The utilization of distillery effluent for microbial biomass production has been reported by several researchers. The growth of *Geotricum candidum*, *Candida krusei* and *Hansenula anomala* either as single or mixed cultures on whisky distillery effluent resulted in about 54.9% COD reduction of the effluent along with good biomass generation [136]. SivaRaman et al. [137] isolated a *Candida utilis* culture and used it for production of single cell protein from distillery effluent. The culture also reduced the BOD of the effluent by 83%. Various organisms such as *Sccharomyces cerevisiae*, *Brevibacterium flavum*, *Paecilomyces variothi*, *A. niger*, *Rhizopus nigricans*, etc., have been grown in the effluent to get protein rich feed [8].

Interest in microbial surfactants has been steadily increasing in recent years due to their diversity, eco friendly nature and the possibility of their production through fermentation. Their potential applications in crude oil recovery, health care, food processing industries and in environmental protection like bioremediation of sites contaminated with poly-chlorinated biphenyls and heavy metals has further increased their scope [138]. Sudhakar Babu et al. [139] performed batch kinetic studies on rhamnolipid biosurfactant production from a *Pseudomonas* strain using distillery waste as substrate. Dubey and Juwarkar [140] demonstrated the production of an effective biosurfactant from *P. aeruginosa* strain BS2 using distillery effluent as a substitute for nonrenewable resources. The isolated biosurfactant possessed potent surface active properties, as it effectively reduced the surface tension of water from 72 to 27 mN m^{-1} and formed 100% stable emulsions of a variety of water-insoluble compounds. The effectiveness of the biosurfactant was also evident from its low critical miceller concentration (0.028 g L^{-1}). Current world wide dependence on fossil fuels for plastics manufacture, scarcity of space for disposal and growing environmental concerns over non-biodegradable synthetic plastic have fuelled research towards development of eco friendly biopolymer material [141]. Hence, attention has been laid on production of poly-β-hydroxybutyric acid (PHB), one of the most extensively studied PHA, produced by bacteria as storage granules providing food energy and reducing power. Son et al. [142] reported the growth associated production of poly-β-hydroxybutyrate from distillery effluent by *Actinobacillus* sp. The enzyme treated distillery effluent enhanced the PHB content. The culture was also found to reduce the COD of the effluent by 60%. In another study conducted by Khardenavis et al. [141] jowar grain based distillery spent wash yielded 42.3% PHB while diammonium hydrogen phosphate supplemented rice grain based distillery spent wash yielded 67% (w/w) PHB, thus proving the potential of using distillery spent wash as carbon source in microbial polymer production. Yamasaki et al. [143] utilized Shochu distillery waste for production of polyunsaturated fatty acids like docosahexaenoic acid and astaxanthin which have important applications in nutraceutical, cosmetic, food and feed industries. A marine thraustochytrid *Schizochyterium* sp. strain KH 105 utilized the wastewater as nutrient source and accumulated value added lipids as well as reduced COD of the wastewater by 35%. Pant et al. [144] investigated the efficacy of distillery effluent amendment for edible mushroom production. Three species of oyster mushroom, namely *P. florida*, *P. pulmonarins* and *P. sajor-caju* were grown on wheat straw and sugarcane bagasse amended with biomethanated distillery spent wash. Wheat straw was found to be a better substrate than bagasse as it supported high yield. Among the three fungal cultures, *P. florida* gave the highest yield of 238.6% at 50% (v/v) effluent concentration. Enzymes are among the most important products obtained for human needs through microbial sources [145]. Mohana et al. [145] tried to exploit the potential of anaerobically treated distillery spent wash for the production of an important enzyme which has application in the industrial, environmental and food biotechnological sector at some stage or the other. Mohana et al. [145] utilized anaerobically treated distillery effluent for xylanase production by a newly isolated strain of *Burkholderia* spp. under solid state fermentation using wheat bran as the lignocellulosic substrate. This is the first ever report on xylanase production using distillery effluent. Xylanase (cellulase free) production was in the range of 5200–5600 U g^{-1} at optimized conditions.

5. Conclusion

This review indicates that a wide range of biological as well as physicochemical treatments have been investigated over the years for the treatment of distillery spent wash. Biomethanation of distillery spent wash is a well established technology; however research on advance anaerobic treatment technologies has been going on for many years. This research has produced many patented systems that provide a variety of advantages in terms of system efficiency, size, capital cost, treatment flexibility, process stability and operating costs. The research into anaerobic digestion continues with efforts to bring into practice outstanding techniques for ecological restoration.

Biological aerobic treatment employing fungi and bacteria has been investigated essentially to decolorize the distillery spent wash. In all instances, it is found necessary to supplement with additional nutrients as well as diluting the effluent for obtaining optimal microbial activity and eventually optimal results. Consequently there is a need to explore more efficient microbes that can decolorize the effluent using it as the sole source of nutrients without much dilution. Physicochemical treatment methods are effective in both color and COD removal. Nevertheless the disadvantages associated with these methods are excess use of chemicals, sludge generation with subsequent disposal problems, high operational costs and sensitivity to variable water input. Considering the advantages and the disadvantages of different treatment technologies, no single technology can be employed for absolute treatment of distillery spent wash. Hence, there is a need to establish a comprehensive treatment approach involving all the technologies sequentially. A delve into the various methods employed for

treatment of distillery spent wash, it is felt that the ideal cost effective and commercial treatment scheme should comprise of biomethanation as the primary step followed by physicochemical treatment and concluding with aerobic treatment. Developing such an extensive and effective treatment will give the triple benefit of environmental protection, energy conservation and production of high value compounds.

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