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# Biological methods for the treatment of industrial waste

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#### Abstract

Every year, industries generate millions of tons of waste in liquid, solid, or gaseous forms, containing harmful chemical residues that pose a significant risk to various forms of life. As environmental protection awareness continues to grow, biological remediation emerges as a highly promising, efficient, cost-effective, and eco-friendly approach for eliminating both organic and inorganic compounds. This method involves harnessing the power of microorganisms and plants through biological processes to effectively remove diverse pollutants. The utilization of biological treatment represents an advanced technology that has gained widespread acceptance in the management of various industrial wastes, including those from pharmaceuticals, textiles, dairy, tanneries, and more. This approach relies on enzymatic degradation, absorption, and adsorption phenomena. This chapter provides an in-depth exploration of biological methods, encompassing aerobic and anaerobic processes, as well as phyto and myco remediation. These techniques are employed for the effective treatment of diverse industrial wastes, offering comprehensive insights into their application and efficacy.

Keywords: Biological methods, industrial waste, aerobic process, anaerobic process

#### Introduction

Waste refers to materials found in soil, liquid, and gas forms that are economically unusable. Originating from a range of human activities such as commercial, domestic, construction, industrial, clinical, agricultural, and nuclear, these materials pose a serious hazard to the ecosystem. Among them, industrial waste is particularly noteworthy for its potential to cause significant harm to the environment. Especially, untreated industrial wastes are considered a major source of pollution in air, water, and soil. According to the World Bank's report, global waste will rise by 70 percent on current levels by 2050 (What a Waste 2.0: A Global Snapshot of Solid Waste Management to 2050). According to another report, the total global waste generation was estimated to be around 9.2 billion tons in 2011. On a per capita basis, this equates to approximately 1.74 tons of industrial waste generated per person per year worldwide.

Industries generate a significant volume of waste, encompassing both non-biodegradable and biodegradable materials. The spectrum of industrial waste is diverse, including elements such as undesirable odors, acids, colorants, dyes, surfactants, minerals, oils, metals, pesticides, organic matter, toxic chemicals, and more. These components can elicit various harmful effects on living systems, underscoring the importance of responsible waste management practices within industrial processes. Typical organic waste includes proteins, detergents, urea, carbohydrates, soaps, and fats. These all compounds contain carbon, oxygen, hydrogen, nitrogen, sulfur, and phosphorus. During biological degradation, these all are converted into mineralized forms (i.e., NH<sub>4</sub>, NO<sub>3</sub>, NH<sub>3</sub>, PO<sub>4</sub>, and SO<sub>4</sub>).

The industries contributing significantly to waste generation encompass a wide range, including paper mills, tanneries, dairies, wineries, pharmaceuticals, textiles, electroplating, leather tanning, petroleum, and more. These diverse sectors produce substantial amounts of by-products, contributing significantly to pollution. The disposal and management of the by-products from these industries pose environmental challenges due to the potential presence of pollutants, exacerbating the overall impact on ecosystems and surrounding areas. Effective

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Department of Chemistry, Sri Sai University, Palampur, Himachal Pradesh, India waste management strategies are crucial to mitigate the environmental consequences associated with the byproducts of these industrial activities. Indeed, the proper treatment and management of industrial waste are crucial prerequisites before its release into the surrounding ecosystem. This becomes particularly vital for the overall development of any country. Therefore, there is a pressing need for cost-effective, environmentally friendly, and efficient methods in waste treatment. Implementing such methods ensures not only the safeguarding of ecosystems but also aligns with sustainable practices that are essential for the continued progress and well-being of a nation. The initial phases of wastewater treatment, namely preliminary and primary treatment, effectively eliminate solid and large materials through processes like sedimentation and skimming. The removal of suspended solids involves simultaneous processes such as flocculation and adsorption. However, the challenge lies in the elimination of dissolved or particulate matter, which primary treatment alone may not sufficiently address. To tackle this, secondary treatment, employing biological methods, becomes essential for achieving comprehensive removal of such substances. Secondary treatment serves as the second phase in waste treatment, employing biological processes to eliminate dissolved waste or organic matter under either aerobic or anaerobic conditions facilitated by microorganisms. Biological treatment methods are commonly categorized

based on the availability of dissolved oxygen. In aerobic treatment, utilizing atmospheric O2, organic pollutants or wastes undergo transformation into sludge and CO2. On the other hand, anaerobic treatment results in approximately 95% conversion of organic matter into CO<sub>2</sub> and CH<sub>4</sub>, with the remaining 5% utilized for biomass in the absence of  $O_2$ . The biological treatment of industrial waste is dependent on the characteristics, source, and nature of effluent. The biological method can remove the waste by absorption, desorption, microbial degradation, and degradation. The microorganisms are able to break down the organic wastes under controlled conditions because they possess enzymes (the groups of hydrolases, isomerases, lyases, oxidoreductases, ligases, and transferases) that allow them to use waste as food and convert them to harmless or naturally occurring compounds, which are safe for animal, plant, and human.

Tertiary treatment or chemical treatment involved in the use of chemical additives, react with undesired materials or chemicals and metals that produce a huge amount of chemical sludge, and deposition of this sludge is also a problem. Therefore, recently both biological and chemical method tends to been implemented for the elimination of all toxic or harmful compounds because the sometimes toxic environment does not allow the microbes to grow and sustain. Biological treatment methods are summarized in Fig. 1.

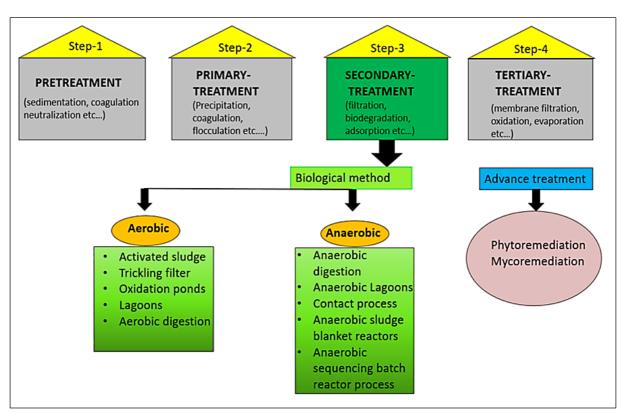


Fig 1: Biological methods for industrial waste treatment

#### 2. Biological Methods

The biological method is applied as a secondary treatment to reduce the organic matter measured as biochemical oxygen demand performed by microorganisms under aerobic and anaerobic processes (Charles *et al.*, 2009) <sup>[21]</sup>. Biological methods have advantages such as (a) affects only targets, no

pollution, and eco-friendly (b) self-sustaining (c) recycling and recovering important components (d) biodegradability capacity (e) efficiently eliminating organic matter (Pinheiro *et al.*, 2019) [94].

Several factors affect the microbes activity and rate of reaction such as pH, oxygen, nutrient concentration, and

(2)

toxic matter and also ensure the growth of microbes under control conditions. The organic matter comprises the energy and carbon source that the microorganisms need to develop, particularly the N and P containing compound. So the microbial degradation efficiency can be improved by balancing the nutrients N, P ratio. The microorganisms are very sensitive to temperature, that can dramatically slow down and speed up the biological reaction rates. The implementation of microorganisms in waste treatment results in the removal of fats, oil and grease, biochemical oxygen demand (BOD) and chemical oxygen demand (COD) decreases and total suspended solids (TSS)

reduction.

The microorganisms involved in processes are divided on the bases of their structure and cellular components: bacteria, fungi, plants and viruses. The microorganisms usefully break down or disintegrate the organic matter using two distinct biological processes: biosynthesis and biological oxidation. Oxidation process results mineralization of end product while biosynthesis converts the colloidal suspension and dissolve matter in to particulate dens biomass (new cells) which further removed by sedimentation process (Fig. 2). Both these process work simultaneously and can be expressed as:

#### (1) Biological oxidation

COHNS (Organic matter) + 
$$O_2$$
 + Bacteria  $\rightarrow$   $CO_2$  +  $NH_3$  + End product + Energy (1)

# (2) Biosynthesis

$$COHNS + O_2 + Bacteria \rightarrow C_5H_7NO_2$$
 (Biomass)

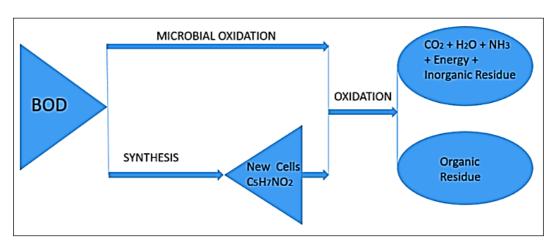


Fig 2: Biosynthesis and oxidation

Biological processes are normally measured the composition and assessed the strength of pollutants in terms of BOD: biochemical oxygen demand, DO: dissolved oxygen, COD: chemical oxygen demand, CBOD: carbonaceous BOD, NBOD: nitrogenous, TBOD: total BOD and SOD: sediment oxygen demand,

Generally, Monod equation used to explain the biological growth:

$$\mu = \frac{(\lambda S)}{(KS + S)} \tag{3}$$

# Where,

 $\mu$  - specific growth rate coefficient, S - limiting nutrient concentration (BOD and COD), KS- Monod coefficient and

 $\lambda$ - maximum growth rate coefficient (0.5  $\mu$ max).

# 3. Biological methods divided in to two processes: aerobic process and anaerobic process

# 3.1 Aerobic Process

The aerobic process is widely favoured for its efficiency, minimal maintenance demands, and cost-effectiveness. Autocatalytic reactions involving aerobic microbes play a pivotal role in the self-purification process, further enhancing the appeal and practicality of aerobic treatment for diverse wastewater treatment applications. These process are biochemically efficient and effectively stabilize the organic matter in presence of  $\rm O_2$  and convert them into  $\rm CO_2$  and  $\rm H_2O$ . This is adequately accomplished by metabolic reaction performed by microorganisms (Fig. 3).

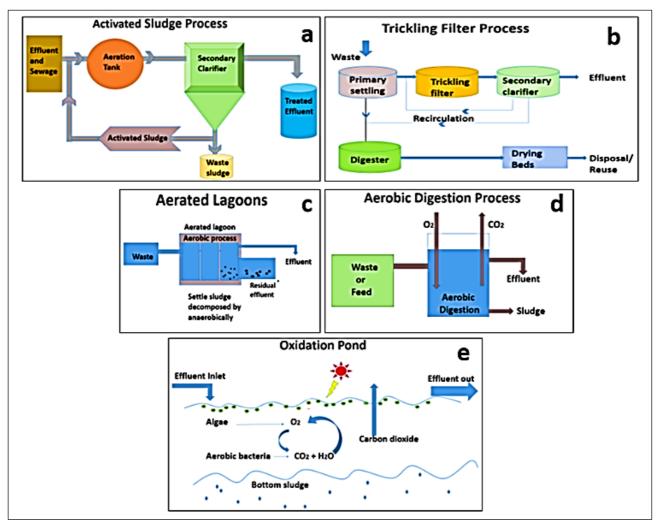


Fig 3: Different aerobic process

Aerobes need free dissolved oxygen in order to decompose organic matter:

$$Organic + O_2 \xrightarrow{Aerobic microorganism} Aerobic microbes + CO_2 + H_2O + energy$$
(4)

The following aspects are to be carefully evaluated for efficient aerobic processes: (a) microbes should be present within the bulk to ensure the biogenesis (b) supply of oxygen to bacteria must be adequate to support aerobic metabolism (c) growth rates must well matched for the different bacteria (d) must be assured the C: N: P ratio required for the growth of microbes (e) establishment of optimal conditions, pH, temperature, nutrients etc. for waste degradation

Aerobic treatment processes reduce effectively harmful gaseous emissions such as CH<sub>4</sub>,  $N_2O$  and ammonia from biowastes reported. Heger *et al.* treat nine dairy farms milkhouse wastewater by the aerobic treatment systems, the results revealed that the oils and grease decreased from 89 mg/L - 9 mg/L while the BOD was eradicating from 539 mg/L - 173 mg/L.

Aerobic process is classified on the bases of working principles, configuration process, oxidation state, feed condition such as: activated sludge process, trickling filter process, aerated lagoons, aerobic digestion process, oxidation pond etc.

# 3.1.1. Activated Sludge Process (ASP)

The activated sludge process is widely used secondary biological treatment method for industrial waste. In 1913 in activated sludge process was discovered by Arden & Lockett at the Davyhulme sewage treatment works in Manchester. This process consists of three phases: first phase is aeration tank, which act as a bio reactor containing mixed microorganism population. Pure oxygen also provided with the help agitation or via diffusers to develop a microbial floc. Second phase a settling tank, that separated the treated waste and solid. The third phase is come back activated sludge equipment, which transmit the settled activated sludge to the aeration tank from the clarifier (Fig. 3a). The continuous mixing of industrial waste or sewage and biological mass ensure proper supply of food to microbes. In aeration tank the concentration of biodegradable waste is decrease and it disperse the mix liquor (mixture of waste and microbial mass) in to sedimentation tank. The estimated sludge in conventional activated sludge process is 0.5-0.8 kg dry weight produce for every kg of BOD<sub>5</sub>. The removal performance of activated sludge is controlled by different parameters such as oxygen supply, temperature, hydraulic residence time in

the aeration tank, surface hydraulic flow, Influent load, concentration of floc and nutrient compositions.

Generally, five groups of microbes that present in the aeration of the activated sludge process: (1) Algae and fungi- is present with pH changes and old sludge (2) Protozoa-Remove & digests the bacteria and suspended particles. (3) Bacteria- remove organic nutrients. (4) Filamentous bacteria-bulking sludge. (5) Metazoa- multicellular organisms that overpower longer age systems carrying lagoons.

#### Principles of operation of activated sludge process:

- a) Activated sludge is the actual process of biological degradation of organic matter where the microorganism converts the harmful waste in harmless product or new cell matter.
- b) In aeration tank the active microbes and waste mix and produce reseeding.
- c) **During the process microbes work under to processes:** adsorbing and absorbing. Oxygen is used to supply the energy for cell growth to produce the final product CO<sub>2</sub> and H<sub>2</sub>O.
- d) **Two microbial species:** floc forming (easily settled on surface of tank) and filament forming do not have sufficient time to settled) composed in system.
- e) System regularly washed out the excess amount of sludge so that the process can be balance.

#### Types of activated sludge process

- a) Conventional complete mix activated sludge process
- b) Plug flow system
- c) Tapered aeration
- d) Step feed AS process
- e) High-rate ASP
- f) Extended aeration
- g) Contact stabilisation
- h) Contact stabilisation
- i) Deep shaft process

The activated sludge aerobic process can be degraded most of industrial waste included: pesticides, soap or detergents, organic chemical, surfactants, polymers, food stuff etc.

In 1980, Kanagawa and his co-workers degrade the pesticide by activated sludge process using D, D-Dimethyl phosphorodithioate (DMDTP). The accommodated activated sludge degenerated in 7 hr (500 mg/liter of DMDTP) and produced inorganic orthophosphate and sulfate 260 mg/liter and 510 mg/liter, respectively. The pH of the mixed liquor was from 6.5 to 7.0. The activated sludge conformed to DMDTP degenerated dimethyl phosphate, dimethyl phosphorothioate, diethyl phosphate and diethyl phosphorodithioate (Kanagawa et al., 1980) [57]. Zipper et al. reported the removal of mecoprop, dichlorprop, and 2,4-D and results revealed 86-98% metabolization of pesticides within 7 days in aerobic conditions. Zipper et al. (1996, 1998 and 1999) further performed the degradation of lindane and chlorophenol pesticides showed similar results. reported on the degradation Another study organochlorinated, cyclohexanes and phenoxy. Pesticides shows not degradation by activated sludge. It has been observed that in sludge the accumulation of pesticides is the biggest risk for microbial fauna. The complete metabolization (86-98%) of copper sulfate, cyprodinil, cymoxanil, diquat, dimethomorph, fludioxonil, folpel,

glyphosate acid, mefenoxam, mancozeb, paraquat and pyrimethanil have been studied within 7 days.

Mizuki *et al.*, reported the degradation of a soap-based fire-fighting agent (SFFA) under aerobic conditions. The batch respirometric tests clearly revealed that within the first day of incubation the three substrate fragments were found to degrade: long chain fatty acid salts, (N, N-bis (carboxymethyl) glutamate tetrasodium salt, and glycols.

Esteve *et al.* studied the deterioration of the thirteen pesticides by using an activated sludge aerobic process. The studies showed that the aerobic treatment remove 97% of twelve pesticides out of thirteen. Additionally, observed that to maintain biomass floc must have added flocculation. Christian *et al.* applied activated sludge reactor with 81-92% of removal waste, where organic loading in between 3 and 85 kg COD/m<sup>3</sup> d with time 2 hours and 2 days.

Guo *et al.* reported the nitrogen elimination with nitrate and the nitrification-denitrification showed thet DO of 0.65 mg/L, 50 to 66 days was the sludge retention time and nitrate collection was 95%. In Egypt, Mourad *et al.* studied and measured the Ra-226 and Th-232 isotope discharge in the environment by phosphate fertilizer plant. Beline *et al.* studied the biological reactor removed 60-70% nitrogen by treating piggery wastewater under nitrification/denitrification. They also observed that by mechanical separation of phosphorous with 80% removal was achieved with bacteria removal from soils.

Gouider *et al.* reported the removal of fluoride and phosphate removal, they found that 97 -98% of fluoride removed from a hydrofluoric acid/phosphoric acid and 93 - 95% from a hexafluorosilic/phosphoric acid.

#### 3.1.2. Trickling Filter Process (TFP)

Trickling filter is a artificial down flow packed bed type of reactor which is widely used aerobic biological treatment system. Trickling filter also well known as percolating filter or sprinkling filter or biofilter (Fig. 3b). The artificial bed consists of various inert materials (porous materials such as coke, slag, rocks, peat moss, ceramic, pumice stone, polyurethane foam, lava, gravel or plastic), which allowed the waste to trickle or sprinkle on the surface. Oxidation of organic matter occur under aerobic condition with the formation of a zoolial film. Effective performance of the trickling filter is indicating by the separation of effluent sludge flocs settling banks.

Biofilm is formed on the surface of inert material and oxygen is provided by the working of intermittent filter. The colour of biofilm is greenish, blackish and yellowish that consist of algae, fungi, bacteria, protozoa etc. Generally, the trickling filters consist of two type of filters: standard rate trickling filters (hydraulic loading of 525 to 2100 m/h/per hectare and organic loading varies from 80 to 400 g/day/m³). The trickling filters having high rate (hydraulic loading of 4200 to 15000 m³/h/ hectare and organic loading varies 400 -4800 g/day/m³). The high rate trickling filters have greater competency than the standard rate trickling filters.

#### 3.1.3. Aeration Lagoon

Aeration lagoons is one of very effective, low cost aerobic waste treatment method. This treatment process equipped with earthen lagoon with mechanical aerators which to promote the biological oxidation and to arrest the settling of suspend biomass (Fig. 3c). Aeration provide the require

oxygen to the metabolizing microorganisms and help to the dissolved and suspended matter with microbes (ASCE, 1988)<sup>[10]</sup>.

Aeration lagoons have capacity of producing effluents below 10 mg/L BOD, TSS. Aerated lagoon shows the significant nitrification in month of summer due to dissolved oxygen limitation. Nitrification of ammonia and BOD removal occur simultaneously. Oxygen requirements for nitrification are more insistent than for BOD removal. Generally, presume that  $1.5~{\rm kg}$  of oxygen is needed to treat  $1~{\rm kg}$  of BOD and theoretically  $5~{\rm kg}$  of  ${\rm O}_2$  are needed to convert  $1~{\rm kg}$  of ammonia to nitrate.

Aerated lagoons are divided according to microbial mass of solid in system: a) Suspended growth aerated lagoon and b) Facultative aerated lagoons.

**Suspended growth aerated lagoon:** Suspension mixed aerated lagoons are shallow earthen basins with 2-5-meter depth and flow through activated sludge have mixed liquor in lagoon. The main objective of these lagoon to converts the biodegradable organic matter in the biomass influent, made then to settle as a sludge. The system is completely aerobic with high aeration ability to keep the solid in suspension.

**Facultative aerated lagoons:** Facultative aerated lagoons are also called partial mix lagoons and where detention time depending up on water temperature. These lagoons are constituted with depth up to 6 meter and ensure the sufficient for oxygenation. The lower part of lagoon is consisting of anaerobic layers and upper part is aerobic layer. Generally, the lagoon consists of three cells, where first cell has most intense aeration, second cell have small aeration, while third cell have very small or no aeration, where the sludge can settle. Facultative aeration lagoon provides about 3.7 to 4 kg  $O_2$  /kW-hour and approximately 70-80% degraded the waste. Diffused systems are more efficient but require greater installation and maintenance effort.

#### 3.1.4. Aerobic Digestion Process

Aerobic digestion is a biochemical oxidative stabilization that uses aeration to minimize organic waste. This method operates on similar principle as the ASP and capable of handling the activated waste, primary sludge and trickling filter and mixture. Under aerobic conditions the organic material is oxidized and products nitrate, phosphate, carbon dioxide, water and lower molecular weight organic compounds. In addition, aerobic digester reduces biodegradable solids content, reduce odours and to make ready the sludge for final disposal on land (Fig. 3d). During the treatment process the waste sludge consists of suspended solids and solids that are production of biomass.

In this process microorganisms utilize oxygen for degradation of organic matter. However, during the inadequate oxygen supply, then the microbes performs endogenous respiration where they begin to consume their own protoplasm for energy. During the process approximately, 75 to 80% of cell matter oxidized and rest converted in non-degradable components.

The oxygen provided for microbial metabolism by either diffused aeration system or through mechanical aerators in digester units. Waste sludge feed line present in each tank, above has the high water level, bottom of the tank contains a solids line and supernatant multilevel line to discard the liquor from the upper half of the tank. To maintained the efficient aerobic environment during the process approximate 1.0 mg/l DO level is desirable. The system set up by feeding raw sludge continuously with supernatant liquor (clear liquor) and digested sludge eliminations. The digested solids are aerated during filling continuously after the tank full. The aeration process is close down for 1 to 2 hours to settle the solid and supernatant liquor. The operation of aerobic digesters is controlled or monitored by various important factors such as: characteristics of waste sludge, temperature, requirements oxygen, mixing, pH and retention time of solids.

Actually, aerobic digestion consists of the oxidation of microorganism cellular matter by organisms and the direct oxidation of the organic matter. The two step reactions are illustrated below:

Cellular material  $+ O_2 \rightarrow Digested sludge + CO_2 + H_2O$  (endogenous respiration) (5)

Organic matter  $+ O_2 \rightarrow \text{Cellular material} + \text{CO}_2 + \text{H}_2\text{O}$  (6)

Aerobic digesters can be performed by batch- or continuousflow reactors.

**Batch Operation:** Batch operation performed physically and the solids are pushed with the help of pump from clarifiers to the aerobic digester with continuous diffused-air aeration. When the all the solids matter is eliminated from the digester, the aeration is discontinued during the removal of the solid from digester and let the solid settle down. The supernatant is then decanted.

**Continuous Operation:** Continuous operation fill-and-draw and the principle almost same to the ASP. The solids are pushed from clarifiers with the help of pump to the digester tank. The digester sent the content in to a solid-liquid separator and balance the return sludge concentration and supernatant standard. Condensed and sustained solids are either reprocessed or removed for additional processing.

The aerobic digestion has various advantages such as:

- a) Easy to operate and capital cost generally low.
- b) Construction cost low.
- c) Process generate low suspended solids, supernatant liquor moderate in BOD<sub>5</sub> and ammonia nitrogen.
- d) Produce end product odourless, humus-like, biologically stable.

The aerobic processes are energy intensive, high operating costs and have excessive waste sludge volume which need extra disposal. Javaid *et al.* reported that dichlorinated pesticides were digested by aerobic process. The studies revealed that ether bond oxidation and cleavage and of the hydroxylation of the chlorophenol to form chloro-catechol. The compound was degraded in water and carbon dioxide by bacterial metabolism.

Liu *et al.* treated sewage sludge by batch-mode operation using auto thermal thermophilic aerobic digestion with effective volume 10 m³. The process attained good VS removal 41.2% within 360 hours. The studied revealed that COD and NH<sub>4</sub><sup>+</sup>-N quickly expanded till 144 hours, and then decline.

# 3.1.5. Oxidation Pond

Oxidation ponds one of well-known biological aerobic process that have been extensively used in remedy of

industrial waste. In 1901, a very famous Mitchell Lake was constructed by the city of San Antonia, Tex. with 275 ha and 1.4 m average depth. After the success of this lake various country also adopted the ponds as a means of treating sewage. Oxidation ponds also called as stabilization ponds usually 5-6-meter-deep and mainly involves in interaction of microorganism community with settled sludge, raw sludge and industrial waste (Fig. 3e). The ponds are self-sufficient, manage the treatment and efficiently remove the biodegradable organic matter, phosphate nitrogen etc. present in waste.

Earlier, it was accepted that the treatment of wastes in oxidation ponds was the symbiotic activity of bacteria and algae alone. In 1983, United States Environmental Protection Agency reported high rate algal aerobic ponds, where algae maintain the dissolve oxygen under natural light in 30-40 m depth in ponds. Further, various studies have been confirmed the activities of different microbial species such as bacteria, fungi, algae, protozoa and viruses in the oxidation pond. These oxidation ponds are simple, low cost technology with very effective remediation for wastewater prior their release in ecosystem. This method is well known for their high biological oxygen demand; 98 to 99% removal efficiency is possible. This method is highly depended upon to climate and weather condition such as temperature, light intensity, pH and wind speed. The different microbial community found in oxidation pond performed their task by competing with each other. In this process the pond decomposer (bacteria) the organic matter and liberate ammonia, nitrates and carbon dioxide. Further these components are utilizing by algae by the process of photosynthesis and release oxygen constitute different groups of microbes such as algae, bacteria, fungi, protozoa, viruses, etc. The pond bacteria decompose biodegradable organic material and release carbon dioxide. These compounds are utilized by the algae, which together with sunlight and photosynthetic process releases oxygen, enabling the bacteria to breakdown more waste and accomplish reduction in BOD levels. Weidemann and Bold reported the symbiotic behaviour and elucidated the nutritional features of algae, bacteria and fungi.

In past decade in India approximate 30-35 wastewater treatment ponds working in Central Public Health Engineering Research Institute Nagpur, with the loading of 22-440 kg BOD<sub>5</sub>/ha per day on pond area of 1 ha. Gehm and Gellman reported 25 pulp and paper mills adopt this treatment process and operating at pond loadings from 11.4 to 345 kg BOD<sub>5</sub>/ha per day with at least 85% of BOD<sub>5</sub>. In 1966, the National Council for Stream improvement and pulp and paper mills have adopted mechanically aerated waste stabilization pond for treating 2 440 000 m<sup>3</sup> waste per day from 26 mills by same process with 50 % to 95 % BOD removal.

#### 3.2. Anaerobic Process

Anaerobic processes are excessively utilized in industrial wastewaters and organic sludge treatment. In this process microorganisms require less or no oxygen to their live and that absolutely mineralize organic material into carbon dioxide and methane via hydrolysis and acidification. In developed countries the ethane production of anaerobic processes has been significantly utilized as alternative energy source for decades.

The anaerobic process involves in two stages and both are in dynamic equilibrium: acid fermentation where anaerobic microorganisms break down complex organic compounds into simpler, short-chain organic acids and methane fermentation consist of two phase: acetogenesis (anaerobic microrgamisms convert organic acids to acetate, hydrogen gas, and carbon dioxide) and methanogenesis (anaerobic microorganisms convert new molecules into methane gas and carbon dioxide). The anaerobic processes included in treatment of organic waste: food processing industries, breweries industries, chemical industries, dairy wastewater, pharmaceutical waste, sugar processing waste, pulp and paper industries waste etc. The produced residue is stabilized, odourless and a good fertilizer, which is beneficiary to boost the crop yields. In anaerobic process some factors that determine the removal efficiency of biodegradable organic matter are included as:

- a) Composition of the organic matter to be removed
- b) Environmental factors suitability
- c) Sludge retention time
- d) Mixing intensity
- e) Contact time between bacterial biomass and organic
- f) Specific loading of organic matter.

#### Anaerobic processes have many advantages such as

- a) Need less energy to degrade the complex chemical compound
- b) Produces low sludge which is less harmful for environment
- c) Biogas and heat use as a renewable energy source
- d) Unrestricted by the oxygen transfer rate
- e) Less biomass generation due to sludge disposal and treatment is reduced significantly.

# Types of anaerobic wastewater treatment systems include the following:

(a) Aerated Lagoons (b) Anaerobic Sludge Blanket Reactors(c) Anaerobic Sequencing Batch Reactor (d) Anaerobic Contact Process

Feitkenhauer and Meyer reported the commonly used sulphonate-based surfactants under aerobic and anaerobic condition. The studies revealed that in anaerobic conditions the sulphosuccinates showed high biodegradation and linear alkyl sulphosuccinates were perfectly mineralized. However, branched alkyl sulphosuccinates showed 50% biodegradation. (Feitkenhauer and Meyer, 2002) [37]. Alkyl sulfates with C<sub>8</sub>-C<sub>18</sub> alkyl chain are classified as anaerobic biodegradable on the DID. The studies showed that as increased the branching of the alkyl chain results in decrease of biodegradability. The anaerobic biodegradation of LAS to SPC was confirmed in laboratory studies with anoxic marine sediments spiked with 10-50 ppm of LAS (Lara-Martin et al., 2007) After 165 days, up to 79 % of LAS was degraded via the generation of SPC. The generation of mineralization products was not determined. Since the degradation rate was rather slow, its impact on anaerobic environmental fate of LAS is still unknown.

Alkylamido betaines are ultimately biodegradable under anaerobic conditions even at high concentrations up to 300mg/L carbon. At the highest test substance concentration an initial inhibition of the biogas production was observed for about four weeks.

# 3.2.1. Anaerobic Digestion Process

Anaerobic digestion or degradation is a multistep process of parallel reaction of waste mixed with active microorganism for organic matter degradation in absence of O<sub>2</sub>. Anaerobic digestion is extensively used source of renewable energy. Digester can have performed as batch process or continuous process. The objective of anaerobic treatment to minimize high organic loads to a BOD level (Fig. 4).

The process of anaerobic degradation can be described as:  $C_cH_hO_o\,N_n\,S_s+H_2O\!\!\rightarrow cCH_4+(c\text{-}x)\,CO_2+nNH_3+sH_2S$  (8) The metabolic pathway of anaerobic digestion can be broken down is different stages shown below (Fig. 5).

**Stage-1:** In hydrolysis the bacteria such as cellulolytic, lipolytic, and proteolytic split the covalent bonds into chemical reaction and organic matter components such as

carbohydrates, lipids and proteins into monomers.

**Stage-2:** In acidogensis stage the acidogens translate the monomers into volatile fatty acids (Khanal, 2008) [59] and also produce alcohols, hydrogen and carbon dioxide. The bacteria that including in this step are *Clostridium spp.*, *Actinomyces, Bifidobacterium spp.*, *Corynebacterium spp.*, *Staphylococcus, Desulphovibrio spp.*, *Lactobacillus, Peptococcus anaerobus*, and *Esherichia coli*.

**Stage-3:** In acetogenic stage the microorganisms translate the volatile fatty acids and ethanol into acetic acid and CO<sub>2</sub>. Stage-4: In methanogenesis stage: Methanogenic bacteria by using anaerobic processes convert acetic acid/acetate into methane.

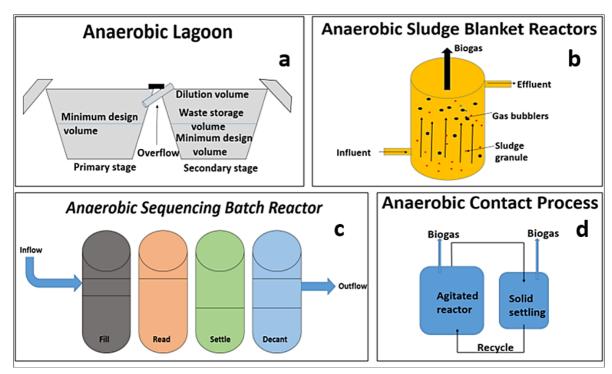


Fig 4: Different anaerobic process

The various factors related with waste, impact the methane production rate such as waste composition, particle size and organic loading rate. Rending the waste can enhance the rate of production of methane and also increase the surface area for bacterial attack. Deublein and Steinhauser, 2008) [28] reported the by the rending of substrate increase the yield up to 20%. The amount of methane that generated during the process is affected by various environment factor including: temperature, nutrient content, moisture content, concentration of toxic substances etc.

# 3.2.2. Anaerobic Lagoons

Anaerobic lagoons are most often process to treat the industrial waste (settle solid, and reduce in soluble organic substrate). Lagoons are a deep earthen basin where anaerobic bacteria break down the pollutants in the absence of O<sub>2</sub>. Anaerobic lagoons are not heated, aerated and mixed, they are very effective in warm temperature (Fig. 4a).

Basically, anaerobic lagoons are designed 8 to 15 feet depth with inlet, outlet flow and treat the waste for 20 to 150 days (EPA, 2012). Inside the lagoons the soil material separate and settle in layers, the top layer consists of greases, oil and microbial metabolism. The organic loading rate reported for

anaerobic lagoons have been varied from 54 to 3000 pounds of  $BOD_5$  per acre per day and elimination percentage ranging from 50%-90%. Lagoon system emitted the substances through by two ways: gas emissions and lagoon overflow. Gas emission is the product of manner and continuously release the gas. The lagoon overflow produces harmful substances such as estrogens, pesticides, heavy metals, protozoa etc. (Tishmack, 2011) [115]. Anaerobic lagoon design is construct on the following remuneration such as: operating levels, site investigation, land application, irrigation equipment, shape, loadings, sludge removal, volume, and solids separation.

A lagoon accomplished their functions in different zones. During the operation performance, minimum level should not drawdown to maintain the treatment and sludge deposition functions. Additionally, to prevent the liquids overflow liquid level must be low. Some factors which determine the predominance of biomass in various zone of anaerobic lagoons are pH, temperature, organic load, nutrients availability, degree of mixing and solar radiation (Pfost, 2011) [93]. Anaerobic lagoon has advantages as following:

- Stabilization of waste, minimizes odour and manure utilize as fertilizer.
- b) Low cost storage of manure for long time.
- c) Using flushing system, manure manipulated with water.

#### 3.2.3. Anaerobic Sludge Blanket Reactors

Anaerobic sludge blanket reactors consist of small agglomerations of microorganisms (microbial granules, 1-3 mm). anaerobic microbes form a blanket of granular sludge and convert the waste into biogas which consist of carbon dioxide and methane (Fig. 4b).

Upflow anaerobic sludge blanket reactors have attained considerable success and applied on various range of industrial effluents such as sugar, chemical, yeast production, soft drinks, slaughterhouse, pulp and paper, dairy, coffee processing, and fish processing industries. In the late 1970s, Lettinga *et al.*, developed the upflow anaerobic sludge blanket process. The different factors effect on particle removal during reactor operational parameters are particle size distribution, organic sludge bed characteristics, loading rate, temperature, up flow velocity, hydraulic retention time etc.

Wastewater introduced at the bottom of the reactors and follows the upward flow through blanket of activated sludge, which are granule aggregates. Granules provides an effective treatment as they have good stability and do not wash out during treatment process. At specified intervals of time a fraction of the sludge withdrawn from the blanket and treated before land application. ASBR produce from 0.1 to 0.2 kg of dry sludge per m<sup>3</sup> of treated wastewater.

UASB reactors is normally shows pathogen reduction for bacteria between zero and 2 log10 units and for other pathogens less than 1 log<sub>10</sub> unit. Helminth eggs removal is the one of most popular pathogen. Another studies reported on removals of helminth egg ranging from 0.42 to 0.92 log<sub>10</sub> units. Pant and Mittal reported Salmonella spp. removal  $0.94 \log_{10}$  Shigella spp., for  $0.78 \log_{10}$  and Vibrio spp. for 0.87 log<sub>10</sub>. In Egypt, utilized UASB reactors to the study the efficiency of fecal bacteria and observed the removals of more than 1 log<sub>10</sub> for total and *Thermotolerant* Pseudomonas aeruginosa, Listeria monocytogenes, Fecalstreptococci, Salmonella Staphylococci. Upflow anaerobic sludge blanket reactors includes several advantages such as:

- a) High reduction of BOD
- b) high organic and hydraulic loading rates
- c) Low sludge production
- d) small land requirement
- e) low construction and operating cost

#### 3.2.4. Anaerobic Sequencing Batch Reactor Process

Anaerobic sequencing batch reactors (ASBR) is early 1960s developed technique with high rate anaerobic process. ASBR is promising solution, which can save considerable energy and utilized for the wastewater treatment including amounts of particulate organic material in different industries (Fig. 4c).

The ASBR process operating under cyclic steps including: feed, settling, drawn and reaction. The first step consists of introduction of substrates with continuous mixing of content. In second step, reaction stopped with mixing of the contents and biomass floc and settlement. The time needed for the completion of reaction involves in biomass concentration, type of biomass, effluent quality and

substrate characteristics. The ASBR has shown promising and extensively high capability for both COD removal and hydrogen production. At the end decantation takes place and microorganisms having low sediment features are also discarded from the reactor.

ASBR calculated the hydraulic retention time for an is as follows:

$$HRT = \frac{V_{ro}}{V_c} X R \tag{9}$$

HRT - hydraulic retention time- days, R is cycles per day (day<sup>-1</sup>), Vc is cycle volume (m<sup>3</sup>),  $V_{ro}$  is tractor operating volume during react phase (m<sup>3</sup>). Some advantages of ASBRs (Xiangwen *et al.*, 2007)

- a) In fixed-bed continuous systems, no short circuit.
- Very high efficiency for both gas production and COD removal.
- c) No need of primary and secondary settles.
- d) Operation flexibility and control system.
- e) Cost saving

Gregor *et al.* reported the treatment of brewery slurry under ASBR system using different organic loading rates from 3.23 to 8.57 kg of COD/m<sup>3</sup> day of reactor. The results revealed 79.6% to 88.9% COD degradation efficiency and for the control, efficiency was noticed 65%. The methane yield was calculated 371 to 418L/kg COD and for control, methane yield was 248L/kg COD observed.

Rahayu and Purwanto studied the ability of ASBR reactor for waste treatment of tofu production industry. The results showed that the active sludge generates accumulative volume of 5814.4 mL at HRT 5 days and obtained COD for 0.16 L of CH<sub>4</sub>/g and produce CH<sub>4</sub> and CO<sub>2</sub> containing 81.23% and 16.12%, respectively. Basheer *et al.*, reported the remydation of slaughterhouse wastewater by utilizing the ASBR and they observed 70%-75% of removal efficiency of COD at different hydraulic retention times. There studies also revealed the stable biogas production at 4.5-8 L/day, 60%-70% methane between 2 and 5.2 kg COD/m<sup>3</sup>.

Cheong et al., studied the stability and high efficiency of the ASBR for treatment of organic waste batch reactor. The observed results revealed higher efficiency of COD removal 86-95% of the system. Xiangwen et al., reported the treatment of brewery wastewater, and the results showed 90% COD removal with the organic loading rates (1 to 5 kg COD/m<sup>3</sup> d). The studies also revealed that within 60 days the sludge granulations were achieved. The studies also concluded 90% of COD removal was attained during fluctuation of volatile fatty. Anaerobic sequencing batch reactor (ASBR) was utilized to treat the tannery wastewater at different organic loading rate (1.03, 1.23, 1.52 and 2.21 kg.m-3.d-1). The results confirm 69-85 % COD removal efficiencies and methane yield between 0.17±0.2 and 0.30±0.02 m3/kg COD removal were observed. Schneider and Topalova study the effective ness of ASBR method for dairy wastewater treatment and confirmed the organic removal was achieved 60% for protein, 70% for COD, and 97% for lactose.

#### 3.2.5. Contact Process

Anaerobic contact process is essential anaerobic activated sludge process that consists of an agitated reactor followed by settling tank for recycling. Anaerobic contact reactors contain external clarifier for the settlement of solids and recycle them to the reactor tank back. Basically, the contact process is essential to thoroughly mixing of the digester contents such as, sludge recirculation, gas recirculation, continuous or intermittent mechanical agitation (Fig. 4d).

The system maintains high concentration of biomass and have better contact with substrate. Degassifier helps the removal of CO<sub>2</sub>, CH<sub>4</sub> (biogas bubbles) from the sludge that may be otherwise drift to the surface. The concentration of biomass in reactor depending up on stability of settled sludge, varies from 4-6 g/L with maximum concentration as 25-30 g/L. The COD loading rate varied from 1 - 8 kg/m<sup>3</sup> /day percent removal efficiency of approximately 85 - 95 %. This process is very much efficient for digestion materials with high suspended solids and successfully retaining flocculent (non-granular sludge) at appropriate biomass level.

# 4. Myco remediation

The termed mycoremediation was invented by Paul Stamets and refers to use of fungi for industrial waste remediation. Mycoremediation is an innovative method and plays an important role in complete discolouring and detoxifying various noxious material in the environment including heavy metals, pesticides, aromatic amines, lignin and dyes, hydrocarbons, cellulosic materials, derivatives etc. Due to easily colonize the fungi eliminate large varieties of waste by utilizing hazardous compounds of some of waste as nutrients source and mineralize or fragmenting into non-toxic substances. Generally, fungal cell walls contain 80-90% of polysaccharide content and other components such as lipids, proteins, inorganic ions and polyphosphates. The distinction in cell wall contents can cause huge variation in metal ion-binding capacity (Fig.5). The studies demonstrate that the fungi eliminate or degrade the contaminants that available in air, soils, or water by some mechanism such as bioaccumulation, biosorption, biotransformation, biodegradation, bioseparation, and biodetoxification. Mycoremediation has some advantages such as:

- a) Naturally occurring process
- b) Eco-friendly approach and non-toxic
- c) Profitable and less invasive Requires zero maintenance and recyclable

Several fungal species are reported for heavy metal elimination such as Aureobasidium pullulans, Penicillium spp., Aspergillus niger, Funalia trogii, Cladosporium resinae, Ganoderma lucidum and Pleurotus tuberregium. Thippeswamy et al. reported the various Aspergillus spp. to concentrate different heavy metals such as, Cu, Cd, Cr, Ni, Pb and Zn. The groups of fungi such as, moulds, mushrooms and yeasts are possessing metal (Ni and Zn) biosorption remediation. Hassan et al. demonstrated the bioaugmentation of Cr, Cu, As, Fe, Mn in soil using consortia of filamentous fungi. The results of fungal consortia revealed the removal of As, Mn, Cr and Cu were 60 %, 71 %, 77 % and 52 %), respectively. The observed results confirmed fungi bioaugmented soil had the maximum metal bio elimination ability than the unprocessed control soil (p<0.05). Mushrooms involve in biosorption mechanism via mycelia for heavy metals uptake and possess high concentration of metals than vegetables, fruits and crop plants.

Rajhans et al., studied the textile industries dye pollutants by enzymatic degradation using mycoremediation. The results showed the removal efficiency of biological oxygen demand (56.3%), chemical oxygen demand (98.5%), total suspended solids (73.2%), salinity (64%), color (89%) and dye concentration (87%) after 18 h. The LCMS results showed acid orange 10 degradation in two compounds: nitrosobenzene and 7-oxo-8-iminonapthalene-1,3disulfonate. Several studies showed biomineralization and detoxification of textile pollutants (artificial dyes and molasses) by fungus, Geotrichum sp. Wanderley et al. also reported the completely degradation or mineralization of azo dyes into CO.

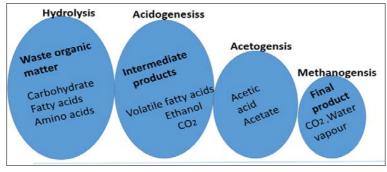


Fig 5: Anaerobic digestion of organic matter

Several studies reported that mushroom can exhibit extracellular peroxidases, xylanases, ligninase, pectinases, cellulases and oxidases. It has been found these enzymes degrade the nonpolymeric pollutants such as nitrotoluenes, PAHs organic, synthetic dyes and pentachlorophenol. Several studies reported that the polymers such as plastics has been degraded by mushroom species.

#### 5. Phytoremediation

Phytoremediation is green eco-friendly sustainable, and affordable technique employs the application of

microorganism associated with plants (aquatic, semiaquatic and terrestrial) for the remediation of industrial wastewater. This method utilizes the plants parts for water consumption, metabolize, remove, degrade or immobilize or detoxification of organic or inorganic contamination (pesticides, Heavy metals, chlorinated solvents, aromatic, petroleum hydrocarbons, crude oil etc. The removal of pollutants influence by concentration of pollutants, plant species, duration of exposure, root system, temperature, pH etc. The phytoremediation mainly accomplished by the growth rate of plants and photosynthetic activity (Fig. 6).

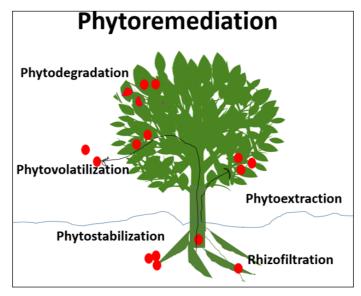


Fig 6: Process of phytoremediation

Bioconcentration factor (BF) help to calculate the total metals uptake capability of growing plants tissues is estimated as:

$$BF = C_p/C_{so} \tag{10}$$

where  $C_p$  is the metal concentration in the plant and  $C_{so}$  is the metal concentration in the soil.

Plants are also competent of translocating and storing elements, the translocation factor (TF) is used to calculate the metal transport as:

 $TF = C_s/C_r$ 

where C<sub>s</sub> and C<sub>r</sub> - metal concentrations (aerial parts and roots), respectively. According to Subhashini et al. the plant transport metals to the shoot by root (indicates TF > 1) and metals are accumulated in roots by immobilization (TF < 1). In plant metabolic reaction help to reduce the waste contaminates by using detoxifying mechanisms, the pollutant enters in plant via. roots, stems, or leaves and adsorb and accumulate the nutrients at contaminated sites and promote the growth. These pollutants changed into less harmful chemicals or changed into gases that are further released into the air. Several aquatic plant species have been reported for wastewater remediation such as Landoltia pinnata, punctata, Pistia stratiotes, Azolla Lemna spp., Spirodela polyrhiza, Marsilea mutica, Riccia fluitans, Eichhornia crassipes, Najas marina, Hygrophilla corymbosa, Hydrilla verticillata, Salvinia molesta, Ruppia maritima, Myriophyllum aquaticum, Egeria densa, Vallisneria Americana, Distichlis spicata, Diodia virginiana, Iris virginica, Nuphar lutea, Cyperus Imperata cylindrical, Typha spp., Phragmites autralis, Justicia americana, Nymphaea spp., and Hydrochloa caroliniensis.

Advantages of phytoremediation of wastewater:

- a) Low capital requirement
- b) Low energy requirement
- c) Environmental friendliness
- d) Utilises natural and renewable source
- e) Less secondary waste generation
- f) Less carbon footprint

Phytoremidation can be achieved through different process phytoextraction, phytodegradation, phytovolatilization, and rhizofilteration, phytostabilization are some techniques used in wastewater remediation shown in Fig. 6.

#### **5.1 Phytoextraction**

Phytoextraction is green process for removal of metallic elements from waste aquatic media. This process utilizes the plants to transport and accumulate the metallic form of contaminates from soil or water into harvestable portion of roots and shoots. Further the harvestable portion simply and carefully operated by drying, ashing or composting. Phytoextraction remediation has been used for accumulation or removal of various metals Zinc, Cadmium, Chromium of Cu<sup>2+</sup>, Pb utilizing various species of plants such as duckwood (Lemna gibba), water spinach (Ipomea aquatic), pondweed (Potamogeton pusillus), Ceratophyllum demersum and Myriophyllum spicatum. Furthermore, Roongtanakiat, studied the heavy metals removal by vetiver from industrial wastewater.

# 5.2 Phytodegradation

Phytodegradation process also call as phyto-transformation which help to break down the different contaminants from the environment, including petroleum, aromatic compounds, volatile compounds etc. by plant metabolic process. The organic compound degradation occurs within plant roots (rhizosphere), where the roots release the enzymes (catalyze and accelerate) and performed the metabolic activities within the plant tissues to release the less toxic substances or simpler molecular forms. Different plant enzymes were observed in degradation of various organic compounds such as, nitroreductaces dehalogenase and oxygenase. Some harm full compounds were successfully degraded or removed by plant species are as nitrogen oxides, sulfur oxides, dichlorodiphenvltrichloroethane. hexachloroethane carbon tetrachloride.

#### 5.3 Phytovolatilization

Phytovolatilization is a process, which includes the diffusion of Volatile organic compounds into the atmosphere by the process of transpiration. Plants consume contaminants from soil and water that travels from the roots to leaves and modified along the vascular system of the plant, in the way and evaporate or volatilize into the air. Limmer and Burken reported the mechanisms which escalation of flux of the

volatile pollutants by the activities of plant roots as following: Water table lowering, gas fluxes advection, boost soil permeability, chemical transport, overprotection toward the surface with water, advection of rainfall and introspection VOCs away from surface. Studies have proven the volatile forms of various organic and inorganic compounds volatilized from plants. Dushenkov, reported the significant up take of radionuclides (Tritium (3H)) from soil selenium composites and take of contain dimethyldiselenide and dimethylselenide by Brassica species.

# 5.5 Rhizofilteration

Rhizofiltration green is technology used for remediation of waste water by aquatic and land plant species. The contaminated water surrounding the plant roots (rhizosphere) and roots absorb, concentrate and precipitate the contaminants. The various studies showed that the several metals such as, Zn, Cd, Cu, Ni, and Cr can be extracted using different plants Indian mustard, sunflower, spinach, tobacco, hyacinth and rye by rhizofiltration. Vasudev *et al.* reported the elimination of uranium from contaminated waste using sunflower (Helianthus annuus) after root contact time of 24 h. Another study showed that the chernobyl contaminated water removed and 90% of Cs and 80% of Sr levels after a contact time of 12 h using similar experiment.

# 5.6 Phytostabilization

Phytostabilization are the processes which helps to the decrease in the bioavailability and mobility of these contaminants through root system (rhizosphere). The Contaminants get attached to the surfaces of plant and are absorbed by the adventitious root system. This process reduces the leaching and increase the environmental protection. Various species of including water lettuce, water hyacinth, duckweeds and smallwater fern have been reported for the elimination of heavy metals from wastewater.

#### 6. Conclusion

Biological treatment is an affordable and environmentfriendly method in industrial waste management since various microbes are allowed to degrade the organic matter under aerobic, anoxic, and anaerobic environments. Biological treatment including various important process activated sludge process, aerobic digestion, anaerobic sludge blanket reactors, anaerobic sequencing batch reactor, anaerobic contact process, etc. performed microbial reaction in absence or presence of oxygen for removal of pollutants industrial wastewater. Wastewater technologies are the integration of various methods such as physical chemical and biological methods depending on the pollutant loading. This review concluded that the biological treatment method is an effective and efficient method for the treatment of industrial effluents especially, anaerobic sludge blanket reactor and anaerobic sequencing batch reactor. Both processes have better performance and high COD removal with a higher organic loading rate. Although, biological methods are environment-friendly, have low cost, and have low by-products generation but for the sustainable and significant outcome, the combined chemical and biological treatment is needed.

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