Indian Journal of Environment Engineering (IJEE)
ISSN: 2582-9289 (Online), Volume-6 Issue-1, May 2026

Economically Viable Nature-based Sewage Treatment Method by Using Life Cycles of Mosquitoes and Non-biting Midges

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Abstract:** The presence of sewage in the surroundings is loathed by humans and animals as it emits an unbearable foul smell. However, many aquatic insects flourish, with their life cycles occurring in polluted water, such as sewage. This is a conceptual paper proposing the use of aquatic life cycles of mosquitoes and non-biting midges in sewage treatment. Sewage is nothing but water contaminated by human faeces and urine, which is mainly organic matter. Theoretical research is conducted to conceptualise a method for producing valuable insect biomass from the organic matter in sewage, so that such a sewage treatment method can earn a reasonable profit on the incurred capital investment after deducting the operating and maintenance costs. The data needed for this conceptual paper are compiled or extracted from the relevant research base/literature available online. The study presented in this paper has found that the proposed sewage treatment process mimics a natural biological process occurring in polluted water. Currently, sewage is considered a waste product that must be treated at a cost before being discharged safely into natural water bodies. It transforms wastewater into a productive resource by deriving value addition in an environmentally friendly and harmless manner. Unlike existing wastewater treatment systems, the proposed process is profitable by selling insect nutrient-rich biomass as an ingredient in fish/poultry feed. It is a bio-energy with carbon capture and storage process if the generated carbon-neutral or bio-carbon dioxide gas is captured and sequestered in a downstream process. The proposed process is part of the circular economy as waste is converted to wealth in an environmentally friendly way without causing air pollution. Each acre of land used, without any water footprint, for the proposed sewage treatment system is productively equivalent to 700 acres of irrigated agricultural land. The proposed sewage treatment plants can also be used to rear large numbers of Wolbachia and sterile male mosquitoes to prevent mosquitoes from acting as disease vectors. The study also finds that further lab-scale research is needed to select suitable species of mosquitoes and non-biting midges, to determine optimal indoor air quality, and to determine the optimal dissolved oxygen in the sewage that can enhance the yield of insect biomass. There is a lack of scientific literature on non-biting midges. India has an ultimate annual potential of 77 million tonnes of aquatic insect biomass from the available carbon-neutral sewage.

***Keywords:** Mosquitoes, Non-Biting Midges, Sewage Treatment, Wastewater.*

Nomenclature:
ACF: Activated Carbon Filter
BOD: Biochemical Oxygen Demand
BECCS: Bio-Energy with Carbon Capture and Storage
COD: Chemical Oxygen Demand
DO: Dissolved Oxygen
MBBR: Moving Bed Bioreactor
PSF: Pressure Sand Filter
STP: Sewage Treatment Plant
TOC: Total Organic Carbon
TDS: Total Dissolved Solids
LEL: Lower Explosive Level
AOB: Ammonia-Oxidising Bacteria

Manuscript received on 27 April 2026 | Revised Manuscript received on 05 May 2026 | Manuscript Accepted on 15 May 2026 | Manuscript published on 30 May 2026.

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I. INTRODUCTION

Untreated sewage or municipal wastewater consists mainly of human faeces and urine, along with wastewater from bathrooms and kitchens. Human faeces contain organic matter, or biomass, including bacteria, viruses, fungi, and parasites that spread cholera, typhoid, diarrhoea, etc., by contaminating water or food. Urine contains dissolved organic matter and is normally sterile without microorganisms like bacteria, fungi, etc, [1]. Sewage treatment plants (STP) are used to reduce the biochemical oxygen demand (BOD) of sewage and to sterilise microorganisms before releasing it safely into water bodies such as lakes and streams [2]. The capital cost of a modern aerobic STP is nearly Rs 30,000 per cubic meter per day, with a total treatment cost of nearly Rs 30 per cubic meter. Nearly 70% of operating costs are spent on electricity, mainly for injecting air into the water to oxidise organic matter in the sewage [3]. The products of an STP are treated water and the separated sludge generated from the contaminated water/sewage, which is a mixture of human excreta and clean water. The primary and secondary sludges can be used to generate biogas, provided that the nitrogen, sulphur, and phosphate contents are within permissible limits [1]. Most existing STPs do not generate any profit, making them commercially unviable for transforming sewage into a valuable resource. Nature-based STPs mimic the natural water-purification process [4]. The first three stages (eggs, larvae, and pupae) of the mosquito life cycle are aquatic, extracting food from water as filter feeders, and the fourth/last stage, as an adult mosquito, is terrestrial. Adult mosquitoes serve as food for land-based birds, reptiles, ants, spiders, etc. In the mosquito life cycle, the adult mosquito emerging from water is nothing but the separation of vivacious organic matter from water to air/land, leading to a substantial reduction in BOD of the sewage. Similarly, the non-biting midges spend the first three stages of their life cycle in water as bottom feeders, with the last stage/ adult midge, as a terrestrial insect.

Mosquitoes and non-biting midges proliferate in sewage containing high organic loads at low dissolved oxygen levels (DO), where other predating aquatic macroinvertebrates are not able to survive [5]. More than 50% of the organic matter or human excreta in the sewage can be converted into protein-rich organic biomass in the form of mosquitoes and non-biting midges, which can be used as fish and chicken feed. Some organic matter in sewage also converts into biogenic carbon dioxide (CO_2), ammonia (NH_3), nitrous oxide (N_2O), hydrogen (H_2), hydrogen sulphide (H_2S), methane (CH_4), sulphur dioxide (SO_2), etc., due to the presence of various bacteria. These gases can also be harnessed by cultivating mosquitoes and non-biting midges in air-tight housings using sewage as a substrate. Utilisation or sequestration of these gases creates a scope to earn extra income or carbon credits. The process offers the valorisation of sewage/human excreta, hitherto considered harmful waste that has to be treated for safe disposal into water bodies, such as streams or rivers, without causing eutrophication and DO depletion. This sewage treatment process is nature-based, as it uses the naturally occurring aquatic life cycles of mosquitoes and non-biting midges that take place in contaminated waters of streams and lakes. This paper explains an economically viable carbon-neutral process and a nature-based sewage treatment method that uses the aquatic life cycles of mosquitoes and non-biting midges.

II. DATA

A. Sewage

Both faecal/fecal dry solids (29 g/cap/day) and urine dry solids (59 g/cap/day) are produced daily in large quantities. Faecal dry mass values have a median value of 38 g/cap/day in low-income countries, as food containing more fibre is consumed [1]. Faeces are composed of water, protein, undigested fats, polysaccharides, bacterial biomass, ash, and undigested food residues. The organic fraction makes up the majority of dry faeces, with nearly 9% by wt. as inorganic salts. Carbon content of faeces is between 44% and 55% of dried solids [1]. BOD values are between 14 and 33.5 g/cap/day, and COD values are between 46 and 96 g/cap/day. The range of dead and living bacteria is 25–54% of the dry solids in faeces, which is why the diseases spread, such as cholera, typhoid, and diarrhoea.

Urine presents less danger to human health due to the absence of living bacteria/ microorganisms. A median volume of 1.4 L/cap/day of urine is excreted, ranging from 0.6 to 2.6 L/cap/day. The median value of dried urine solids is 59 g/cap/day, ranging from 57 to 64 g/cap/day. The dry matter content of domestic sewage is at least 100 g/cap/day when kitchen and bathroom dry waste are included. Per capita sewage generation is nearly 100 Litres/cap/day. The dry matter content is a minimum of 0.1% by weight of sewage generated. Total organic carbon (TOC) of sewage (mg/Liter) exhibits a linear relation with its BOD [6]. BOD of surface water shall not exceed 4 mg/Litre. Industrial, agricultural, and aquatic water use requires BOD to be less than 5 mg/L, and treated sewage should be between 10 and 60 mg/L BOD, depending on treatment grade. The BOD range in urban sewage is 200-800 mg/L [7]. Biochemical treatment of wastewater is feasible when the BOD-to-COD ratio exceeds 0.3 [8].

Anaerobic digestion is suitable when the C: N ratio is between 20:1 and 30:1, which is not the case for a faeces-urine mixture with a C: N ratio of 2.3:1 [1]. Relatively high levels of sulphate, 1.34–1.63 g/cap/day, were recorded in urine, which can be toxic to methanogenic bacteria in anaerobic systems. In aerobic systems, the recommended C: N:P ratio (100:10:1 to 100:5:1) is not feasible. The higher concentration of potassium (K^+) in faeces is highly inhibitive to aerobic systems. Ammonia toxicity from the urine fraction could have adverse effects on biological systems, as most of the nitrogen in urine is converted to ammonia. Thus, there are limitations in treating sewage using aerobic and anaerobic methods, depending on the constituents of faeces and urine. Due to the high-water content exceeding 99% in domestic sewage, it is not economically feasible to implement anaerobic systems to produce methane gas. Nature-based aerobic oxidation ponds are also not economically viable due to the large urban land area required.

B. Mosquito Life Cycle

Mosquitoes belong to the Culicidae insect family. There are more than 404 mosquito species and subspecies belonging to 50 genera and 2 subfamilies (12 tribes) in India. Of the 404 species, only 37 are primary or secondary vectors of human diseases [9]. The rest of the species are either simply blood-sucking (nuisance) or herbivorous. Primary vectors live in the surroundings of human dwellings, whereas secondary vectors live outdoors, like fields, forests, etc., sucking blood from animals, birds, etc. Japanese encephalitis is spread by a total of twenty (20) species belonging to Culex (11), Mansonia (3), and Anopheles (6) as primary and secondary vectors. Lymphatic filariasis, also known as elephantiasis, is transmitted by four (4) species of Culex (1), Mansonia (2), and Aedes (1) as primary and secondary vectors. Three species of Aedes transmit dengue and Chikungunya as primary and secondary vectors. Ten species of Anopheles transmit malaria as primary and secondary vectors. A malaria parasite (Plasmodium vivax) infected mosquito takes nearly 23 days to transmit the disease to humans [10]. While selecting a suitable mosquito species for a nature-based STP to minimise installation costs, the larvae should have a short water life cycle (days), irrespective of their disease-spreading or blood-sucking nature. Also, a preferred mosquito species should breed in a small water volume or water surface area at high population density without indulging in cannibalism. They should also lay eggs on the water surface. Mosquitoes are holometabolous insects that undergo metamorphosis from eggs to larvae, then into the pupal phase, and ultimately into adult mosquitoes, as shown in Fig. 1 [10].

- **Eggs**

Many species of gravid/pregnant mosquitoes lay eggs on stagnant water, which will take 24 to 48 hours to hatch into larvae [11]. The eggs are either small rafts of eggs cemented together or floating individual eggs. Each time, a female gravid mosquito lays 100 to 300 eggs. The favourable water temperature is between 21 and 29 °C [12]. Colder

Retrieval Number:100.1/ijee.A187206010526
DOI: 10.54105/ijee.A1872.06010526
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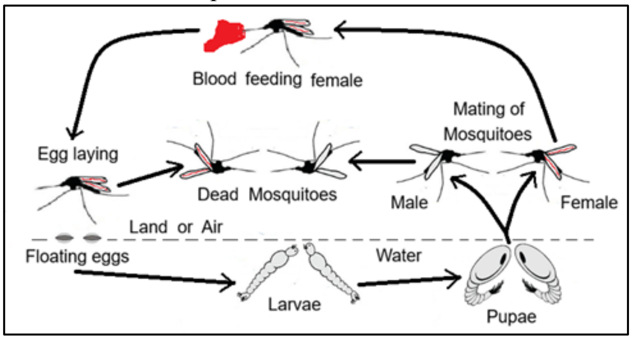
temperatures delay hatching, whereas warmer temperatures are lethal to eggs. Higher humidity levels are favourable for egg hatching [13]. Water with too low DO levels may hinder hatching. A neutral pH is best for egg conversion into larvae [14].

- **Larvae**

Initially, tiny larvae (1st instar) emerge from eggs and begin feeding on nutrients in the water until they become pupae. During their growth, the larvae shed their skin, called moulting, four times. The stages between moults are called instars. These are detritivores or filter feeders on decomposing organic matter, bacteria, fungi, plankton, algae, etc. The larvae's heads are on the underside to enable the brushes on their mouths to filter small particles from the water. They breathe air/oxygen using a snorkel-type breathing tube, called a siphon, located at their tail end. The larvae frequently come to the water surface to breathe air, as they are not sufficiently capable of absorbing DO from the water [15]. Only the larval stage of the mosquito life cycle consumes the organic matter in the water to reduce the organic matter/BOD/COD. Many species of mosquito larvae do not indulge in cannibalism by eating eggs, larvae, and pupae of their species or other species. The pupa emerges when the 4th instar larva moults. The favourable water temperature is 21 to 29 °C with a pH not below 6.5. Larvae can grow healthily in darkness at low DO levels (3 to 4 mg/Litre), where other predators, such as fish and dragonfly larvae, cannot survive. A reduced level of DO does not affect the larvae's growth as long as atmospheric oxygen is readily available [15]. Larvae excrete body fluids that consist of urea and allantoin along with water [16].

- **Pupae**

Suitable water temperature for pupae survival/ metamorphosis is between 20 and 30 °C. Pupae do not feed and rest at the water surface, breathing air/oxygen, though they are highly mobile, diving down through the water to escape any threat. The pupa is lighter than water and therefore floats at the water surface [12]. Pupae convert into adult mosquitoes in one to four days, depending on water temperature and species. When the mosquito pupa is fully developed, the adult mosquito splits open its exoskeleton and emerges into the air. There is an equal chance of becoming male or female mosquitoes.



[Fig.1: Life Cycle of Mosquitoes]

Adult Mosquito

Mosquitoes survive best in humid surroundings. Male mosquitoes take less time to emerge from pupae compared to female adult mosquitoes. Females are generally ready to mate almost immediately after leaving their pupal casings. Male mosquitoes of one or two days old are capable of impregnating the adult females immediately after their birth [17]. Males generally mate with females close to their birthplace and inject seminal fluid containing protein-rich matter to provide the initially required energy to female mosquitoes [18]. Mating in mosquitoes is quick, lasting less than 15 seconds, and usually takes place in the air or on a surface. In some mosquito species, males can grab the female pupa's trumpet or breathing tube, as males can somehow tell which pupa are female and which are male [18]. Females undergo mating only once in their lifetime and store the sperm in their bodies for egg laying many times during their lifespan. However, males can mate repeatedly with many females throughout their lifespans. Both females and males feed on floral nectar. When the female reaches puberty, she needs a protein-rich blood meal to develop eggs. Mosquitoes can suck blood from humans, animals, birds, reptiles, etc. Male mosquitoes also prefer to feed on blood, even when a readily available sugar source is available. Male mosquito life span is reduced to a few days on blood feeding compared to more than a month's life span on sole sugar feeding [19]. Gravid female mosquitoes can be lured to water bodies to lay eggs using semiochemicals [20].

The compound eyes of mosquitoes are well-suited for nocturnal navigation/flying in low-light conditions. The mosquito antenna can detect odours (carboxylic acids, lactic acid, and ammonia related to sweat, and octenol emitted by cattle) to distinguish potential hosts, sugar meal sources, and egg-laying (oviposition) sites. Octenol is a naturally occurring byproduct of oxidation in plants and animals with a diet high in vegetable matter, such as oxen and cows [21]. Humans also emit it to a lesser extent. It also has thermal and humidity receptors. An adult mosquito processes sound through a mechanosensitive organ, or Johnston's organ, which is among the most sensitive sound detectors in insects. This doughnut-shaped organ at the base of the antenna detects minute high-frequency vibrations from sound waves. The air-borne female flaps its wings at 500 times per second, while the male flaps its wings at a lower rate. Conspecific male and female mosquitoes can detect the sound of each other's wingbeats and also adjust their wing speeds to mate during the flight. The antennae of mosquitoes are the primary organs for scent perception. These paired appendages are covered with hundreds of tiny hairs called sensilla, each of which can detect airborne molecules, including various chemical odours emanating from animal skin. An adult mosquito has a pair of sensory palpi next to the antennae, which are crucial for detecting chemicals and carbon dioxide in exhaled air during animal respiration [10]. Carbon dioxide is invariably present in the exhaled air of animals, which is considered a source of potential blood meal by the female mosquitoes. CO_2 is also present in the air (nearly 420 ppm by weight). Malaria mosquitoes can detect changes in CO_2 concentration as little as 0.01%. The Anopheles melas species can detect plumes of carbon dioxide at a distance of 18 metres. Thus, an adult mosquito is a macroinvertebrate with high-order evolution built with sensitive organs for its survival and mass propagation.

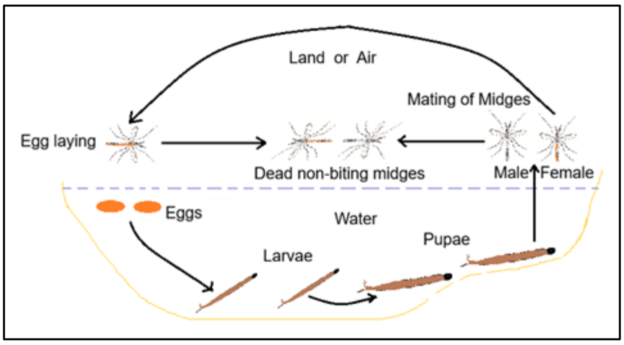
Mosquitoes are found and can survive normally at high altitudes, such as Tibet, where the oxygen level is equivalent to 15% oxygen by weight in air at sea level. Mosquitoes can also be reared in oxygen-depleted air in an airtight housing/enclosure, where oxygen levels can be maintained at around 15%. They can also survive in highly polluted air laden with H_2S, SO_2, NH_3, N_2O, CH_4, etc., gases emitted by foul-smelling water, which is preferred as their breeding site.

- **Mosquito Biomass**

A newly born adult mosquito weighs nearly 2 milligrams (500,000 mosquitoes per kg) [21]. Vacuum-dried mosquito weight is nearly 50% of live mosquitoes, as they contain around 58% moisture. The nitrogen content is nearly 10% by weight in the dried mosquitoes. Mosquitoes contain a higher crude protein content than soya bean meal or fishmeal. Dried mosquitoes also contain nearly 1.8% phosphorus and 1% each of calcium and potassium by weight [22]. Mosquitoes not only reduce the BOD (organic matter) in sewage but also reduce the total dissolved solids (TDS) content in water to a large extent, since the ash content of dried mosquitoes is around 6.5 to 8.5% by wt. As the sewage has high levels of nitrates and phosphorus content exceeding the permissible levels in water bodies, sewage treatment/remediation by cultivating mosquitoes in air tight housing is a superior sewage treatment method for producing protein rich biomass suitable for fish/poultry meal and generating better treated water chemically and biologically without leading to eutrophication (excessive nutrients like nitrates and phosphorus) when treated sewage is discharged into water bodies like lakes, streams, rivers, etc. Mosquitoes are produced by consuming organic matter and dissolved minerals present in sewage. Carbon present in sewage is transferred to mosquito biomass and, during the larval and pupal stages of the life cycle, partly converted into CO_2 by their respiration. The remaining carbon is present as the residual organic matter (below the permitted BOD) in the treated sewage. Nearly 50% of the carbon content in the sewage is transferred to the live mosquitoes when they emerge from the water surface. The carbon content in dried mosquito biomass does not exceed 35% due to high levels of nitrogen (9 to10%) and ash content (7.5%), and normal levels of oxygen (40 to 45%) and hydrogen (5 to 7%).

C. Non-Biting Midges Life Cycle

The non-biting midge flies, belonging to the Chironomidae or Chironomids family, are found in sewage oxidation and settling ponds, rivers, lakes, and ponds that are rich in decomposing organic matter. There are more than 20,000 midge species globally, including on Arctic landmasses and high-altitude plateau regions such as Tibet. Many species of chironomid midges can grow well in water with DO levels up to 3 mg/L [23]. These are also called "blind mosquitoes" because they resemble mosquitoes but do not bite. Adults are attracted to light as they are found in large numbers near street lights, etc [24]. Densities of over 74,000 larvae/square meter are found on the bed of nutrient-rich water bodies [25]. Aquatic stages of midges are bottom dwellers, often found in nutrient-rich, polluted water.



[Fig.2: Life Cycle of Non-Biting Midges]

Chironomid midges undergo a four-stage life cycle like mosquitoes, as shown in Fig. 2. Female midges lay eggs on the surface of the water in the form of a gelatinous egg mass containing 1000 eggs at a time. Eggs sink to the bottom and convert into larvae in about three days when the water temperature is between 25 and 28 °C. Growing larvae enter the suspended sludge/mud or construct small tubes in which they live. The tubes are constructed from sediment/mud and silk secreted by the salivary glands of the larval body. Larvae are opportunistic omnivores and detritivores [26]. They feed on suspended organic matter in the water or mud, regardless of nutrient value, with low digestion efficiency. Larvae also consume ammonia-oxidising bacteria (AOB), thereby increasing NH_3 release from sewage [27]. In their digestion process, larvae excrete copious quantities of faecal cylindrical pellets [23]. Microorganisms slowly degrade faecal pellets into sludge. They are most active in darkness, which protects them from predators [28]. In darkness, larvae come out of their tubular enclosures to feed voraciously. Mature larvae are called bloodworms because the initial pink colour changes to red. The red colour is due to haemoglobin found in the blood of midge larvae. Haemoglobin allows the larvae to survive in low-DO water. Chironomus plumosus, a species of non-biting midges, is the champion in terms of least DO dependency [29]. Under favourable water temperature and nutrient availability, larvae transform into pupae in 2 to 3 weeks.

Pupae live about three days at the bottom and actively swim to the water surface to emerge as flying adult midges several hours later. Adults mate in swarms soon after becoming airborne. Adult midge flies do not feed but drink water or floral nectar and live for 3 to 5 days only. The entire life cycle, from egg to adult, can be completed in 2 to 3 weeks during the summer. The nutrient value of dried midges is comparable to that of mosquitoes [30].

D. Existing STP Technology

Most commonly, municipal wastewater plants utilise the activated sludge process or a moving bed bioreactor (MBBR), which uses aeration and a biological floc composed of bacteria and protozoa. In the process, the organic matter is mainly converted to CO_2, NH_3, and H_2O [7]. When sewage/wastewater, as part of secondary treatment, is aerated in the presence of microbes, the microbes consume the organic matter and multiply rapidly, forming floc/sludge that is separated from the water using a Clari flocculator. Before treating

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DOI: 10.54105/ijee.A1872.06010526
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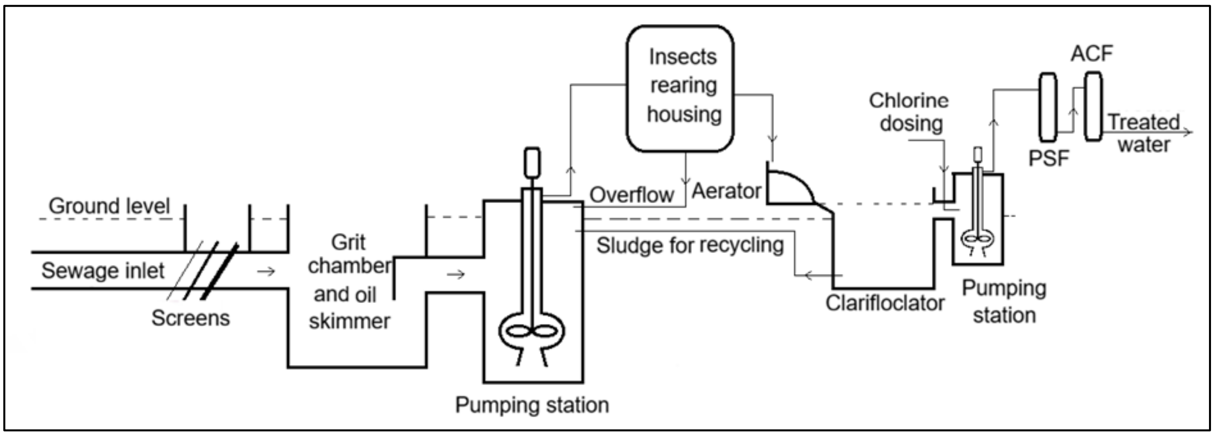
wastewater with microbes in the secondary treatment process, it is screened with a bar/mesh screen to remove suspended particles larger than 5 mm, such as plastic, paper, hair, rags, etc. The screened water is later passed through the grit removal chamber to settle sand, gravel, rocks, and other heavier material. Later, sewage is subjected to primary treatment to remove suspended solids, where the water is passed through a sump/wide channel at very low velocity. The heavier solids settle at the bottom, and lighter solids (oil, grease, etc) float to the water surface for removal by skimmers. The solids extracted from primary treatment are called primary sludge. Primary treatment removes 25-40% of the BOD from wastewater. After the floc/sludge is separated from the water in the secondary treatment, the water is treated with chlorine as a disinfectant to kill any remaining bacteria/microbes. To remove dead bacteria and residual suspended solids, water is initially pumped through a pressure sand filter (PSF) and subsequently through an activated carbon filter (ACF) to produce the required effluent quality (BOD < 60 mg/L) suitable for discharge into natural water bodies [6]. The excess activated sludge/floc (secondary sludge), after recycling to meet process requirements, is dried in a vacuum filtration plant and disposed of as solid waste in dump yards or incinerators [31]. It is also feasible to use mixed sludge, including primary sludge, to generate biogas/methane if the elemental composition of the mixed sludge is acceptable for anaerobic treatment [1]. Since secondary sludge (mainly dead microbes) contains nitrogen, nitrates are also removed along with BOD from wastewater. However, substantial phosphorus removal is not possible. Generally, phosphorus is present in municipal wastewater at levels exceeding permitted limits. Excessive phosphorus content in natural waters promotes algal blooms, which are detrimental to fish growth/survival. Algal blooms reduce water DO levels below the fish survival limit at night by consuming DO for respiration.

III. DISCUSSION

The proposal is to use the aquatic life cycles of mosquitoes and non-biting midges in the sewage treatment process to reduce BOD, nitrates, and phosphates for safe discharge into natural water bodies. In the process, the nutrient biomass of mosquitoes and midges is harvested, which can be used as fish or poultry feed. It is a nature-based solution, as it mimics natural processes [4]. The treatment process is a profitable proposition, as it can earn a return on the capital investment after deducting annual operating and maintenance costs, unlike existing municipal water treatment plants. Moreover, it is a bioenergy with carbon capture and storage (BECCS) process in which the bioenergy available in sewage is converted into valuable nutrient biomass, and the released CO_2 and other gaseous pollutants can be captured and sequestered. In the existing state-of-the-art STPs, greenhouse gases and other gaseous pollutants are released to the atmosphere because it is not economically feasible to capture gases at very low concentrations.

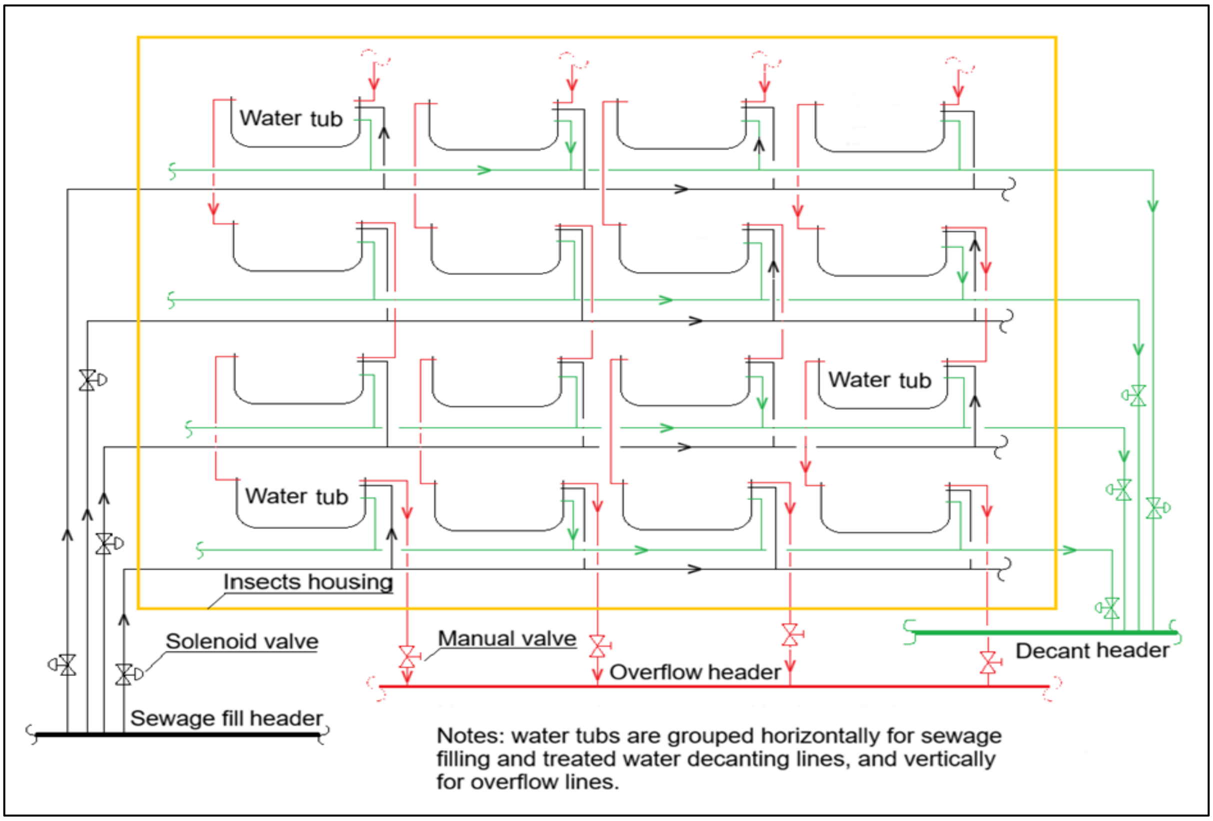
Sewage after preliminary screening/processing is pumped into water-holding tubs to store the water at depths of up to 150 mm. The schematic diagram of the proposed wastewater treatment is shown in Fig. 3. These sewage holding tubs are stacked vertically, one above the other, with individual supporting arrangements, as shown in Fig. 4. Nearly 150 mm vertical spacing is provided between the tubs. These tubs are located in an airtight concrete housing with outer walls. On each floor of the housing, 10 tiers of tubs are stacked one above the other. The airtight housing is 10 floors high to accommodate nearly 100 tubs stacked vertically. Each tub is provided with a sewage fill line, a treated water decant line at the middle of the water depth, and an overflow line that is connected to the bottom tub. Fresh air is supplied to the housing space to maintain optimal O_2 levels for larvae, pupae, and adult mosquitoes. Also, to adult midges as shown in Fig. 5. As the water storage depth of 150 mm is shallow with a huge surface area, the water surface area is adequate for the water to absorb the O_2 from the maintained indoor air quality (16% O_2 by weight) and achieve DO levels between 3 and 4 mg/litre for the growth of midge larvae and pupae [5]. If the DO levels are deemed inadequate, a low-pressure air supply is to be provided to aerate the water in each water-holding tub.



[Fig.3: Schematic of a Wastewater Treatment Plant with Vertical Farming of Aquatic Insects]

For a population of 100,000, nearly 10,000 cubic meters of sewage is generated per day, with 10,000 kg of organic content (i.e., 0.1% by weight). The sewage, after separating the floating debris, grit, and grease/oils/microplastics in the preliminary separation process, is pumped to the tubs located in the airtight housing for rearing mosquitoes and non-biting midges. The larvae of midges and mosquitoes consume the suspended organic solids, the living/dead microbes and the dissolved organic liquids so that the BOD, nitrates and phosphates in the water are either consumed for their biological growth or exhaled as CO_2 and NH_3 mainly, with traces of N_2O, H_2, H_2S, SO_2 and CH_4 gases released by the microbes of all sorts and the larvae. Adult mosquitoes and midges are lured into the housing and killed by a mild microwave treatment. Some mosquitoes (one or two %) are fed with bloodmeal collected from slaughterhouses to develop eggs and lured back to the water tubs for laying eggs. Male and female midges do not need floral nectar for mating purposes and complete the mating process within a day. Females lay eggs in water in the tubs, and eggs float on the water till they hatch. A 1% probability of females laying eggs is sufficient to sustain the midges' biological cycle in the air-tight housing. Midges are attracted to the harvesting area by illuminating the area to kill them with mild microwave exposure. Only 50% of the treated water (75 Litres per M^2 surface area) in a tub is decanted and immediately filled with sewage, leaving the rest of the water (75 Litres per M^2 surface area) as dead storage. Dead storage serves as a temporary refuge for the eggs, larvae, and pupae of mosquitoes and midges. The net sewage holding capacity is only 75 mm of water depth in each tub. In an eight (8)- day sewage retention cycle, 12.5% of all tubs are decanted and refilled with sewage each day to support larval growth. The housing can hold eight days of sewage production, divided into eight or nine independent segments, with one segment at a time for maintenance if required. The population density of larvae and pupae of mosquitoes can be up to 37,000 per square meter of surface area of water, and that of midges is 70,000 per square meter of the bottom area of water. Depending on the availability of nutrients/organic matter in the sewage, the population density and life cycle duration of mosquitoes and midges are determined. When the organic matter in the sewage falls below normal levels, the water retention time can be reduced to maintain an optimal life cycle duration. The treated/effluent water quality is also low in nitrates and phosphates, as mosquitoes and midges take up these elements.

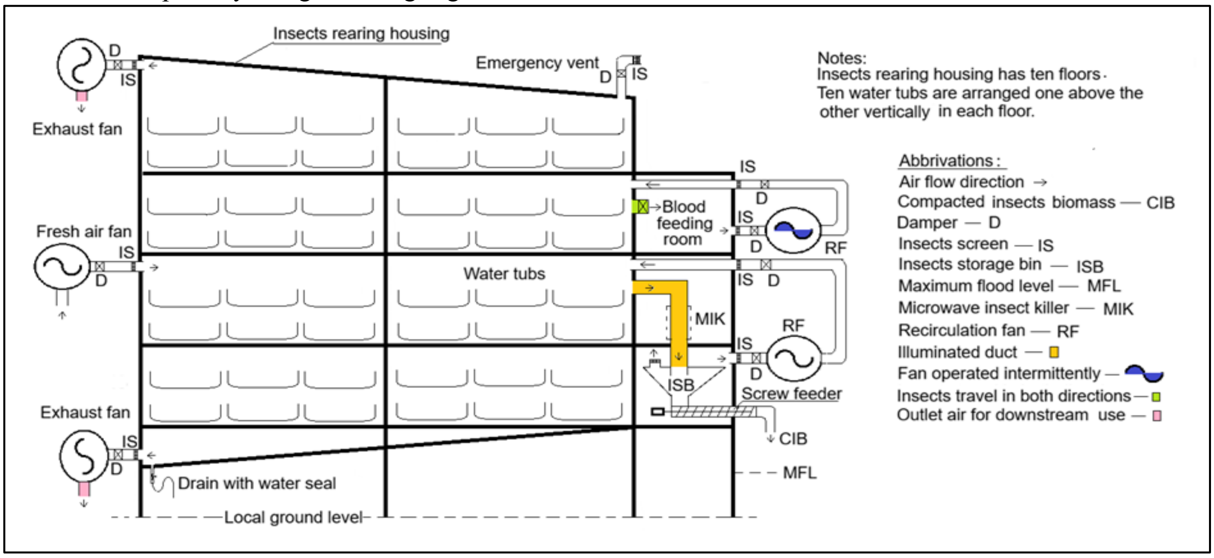
The decanted water from the water tubs is sent to an aerator by gravity to remove dissolved gases and precipitate dissolved iron, and then to a clarifier to remove suspended solids. The effluent water is further disinfected with ozone or chlorine before being sent to PSF and ACF to meet statutory discharge requirements. Ozone dosing is preferred because it enhances DO levels while killing microbes. Chlorine, being a hazardous gas, must be transported by road tankers, whereas ozone can be produced in-house from air using electricity [32]. Any sludge water collected from the clarifier and from PSF/ACF backwashing is mixed with the sewage and pumped to the water tubs. Thus, the proposed process does not generate sludge daily. However, the inert sediment collected in the water tubs is to be removed periodically (every 6 months).



[Fig.4: Hydraulic System of the Housing for a Vertical Farming of Aquatic Insects]

O_2 levels in air-tight enclosures are kept low, up to 16% by weight, to prevent predators like frogs, spiders, bats, lizards, chameleons, etc., from eating adult mosquitoes and midges. The DO level in the water is also maintained between 3 and 4 to prevent the growth of aquatic predators such as nymph dragonflies, tadpoles, and fish, without harming the growth of larvae and pupae of mosquitoes and midges [33]. The species of mosquitoes and midges are selected that do not indulge in cannibalism and eat eggs, larvae, and pupae of the other species. Each segment of the airtight enclosure is nearly 30 m in height with 10 floors. Heavier gases like CO_2, SO_2, and N_2O tend to collect at the bottom, and lighter gases like NH_3, H_2, CH_4, and H_2S at the top of the enclosure due to the stratification effect [34]. Fresh air is supplied to the enclosure so that the gas concentration at any point is below the lower explosive level (LEL), thereby eliminating fire hazards or explosions within the enclosure [35]. Indoor air is purged/extracted from the top and bottom portions of the enclosure to maintain an optimal concentration of each gas tolerable to mosquitoes and midges, and to extract the gases for downstream use. It is expected that the NH_3 concentration would be substantial after the CO_2 gas. Being a polar gas, NH_3 is highly soluble in water (0.36 kg per kg of water), and it can be separated from the contaminated air by water washing. Such aqueous ammonia solution can be used as a cleaning liquid or a fertiliser in nearby agricultural fields. N_2O (a greenhouse gas), SO_2, and H_2S can also be separated from contaminated air by water washing, as they are moderately soluble in water [36]. It is not envisaged to separate and use the primary sludge for biogas generation in anaerobic digesters, as the insect biomass produced from the primary sludge is more productive in terms of quantity and unit sale price. To carry out maintenance jobs by workers inside the housing, all polluted air must be purged out with fresh air before starting the repair work. Suitable nets/screens are provided in the housing ventilation system to prevent the escape of mosquitoes and midges. Also, filter screens are installed in the water lines to prevent carryover of mosquito or midge eggs, larvae, and pupae during the decanting of treated water. All water supply valves are located outside the housing, so there is no need to enter the housing to operate them.

There is scope to extract NH_3, CO_2, and CH_4 in a downstream process through additional profitable investment. Also, nearly 600 kW of rooftop photovoltaic power can be installed. Ammonia- and CO_2-rich air can be used in photobioreactors to cultivate fast-growing microalgae, which is also a superior-grade poultry feed [37]. If the CH_4 concentration exceeds 1% by weight in the extracted air, it can be used as fuel in a lean-burn gas turbine to generate electricity, thereby adding value [38].



[Fig.5: Ventilation System of the Housing for Vertical Farming of Aquatic Insects]

A. Economic Analysis

Total sewage produced = 10,000 M^3/day for a population of 100,000.

Total organic matter in sewage = 1000 mg/Litre

Weight of each live adult mosquito/midge [21] = 2.0 mg

Moisture content in live adult mosquito/midge [22] = 58%

Carbon present in the dry biomass of adult mosquito/midge = 35%

Carbon present in the biomass of live adult mosquito/midge (100 gms of live insects has 58 gms moisture and 35% of the remaining 42 gms is carbon) = 35 x (1-0.58) = 14.7%

Total organic carbon (TOC) of sewage @ 44% by weight [1] = 1000 x 0.44 = 440 mg/Liter

Nearly 50% of the TOC is converted into insect biomass, with 14.7 % carbon content.

Mass of insects generated from sewage = 440 x 0.5 /0.147 = 1497 mg/Litre of sewage.

Insects' biomass that can be generated from the sewage = 1497 x 10000 x 1000 /10^9 = 14.97 tonnes/day = 4491 tonnes/year at 300 days operation

Nearly 5% of TOC remains in the sewage as residual BOD = 440 x 0.05 = 22 mg/Litre

The remaining 45% TOC is converted into CO_2, mainly: 440 x 0.45 = 198 mg/Litre.

O_2 needed for converting TOC to CO_2 gas = 198 x 32/12 = 528 mg/Liter.

O_2 present in the organic matter of sewage @ 45% = 1000 x 0.45= 450 mg/Liter of sewage

5% of organic matter remains as residual BOD in the sewage, with @ 45% of O_2= 450 x 0.05 = 22.5 mg/Litre of sewage [6]

O_2 present (45%) in the harvested mosquitoes and midges = 1497 x 0.45 = 674 mg/Liter of sewage

Net O_2 required in the air supply = 528+674-450+22.5 =774.5 mg/Liter of sewage

CO_2 gas generated = 198 + 528 =726 mg/Liter of sewage

O_2 gas consumed at a minimum 16% O_2 concentration in the indoor air = 80 mg/Liter of air or 8% of air by weight.

Total air supplied = 774.5 / 0.08 = 9681 mg/Liter of sewage

Total indoor air with CO_2 gas = 9681 +726 - 528 = 9879 mg/Liter of sewage

Composition of CO_2 in the indoor air = 726/10407 = 7.34% by weight.

Total air supply needed at very low pressure (below 100 mm water column pressure) = 9681 x 10,000 x 10^{-9} /24 = 4.03 tonne/hour (stoichiometric requirement). However, the actual air supply would need to be a few times higher to maintain proper CO_2, NH_3, etc., levels for the healthy growth of insects.

Power consumption for pumping sewage to a height of 40 meters is 0.15 kWh/M^3

Water surface area for 75 Litres of water storage in tubs = 1 M^2

Sewage retention duration = 8 days

Total area of storage in tubs = 8 x 10,000 x 1000 /75 = 1,067,000 M^2

Land area required = 1,067,000 / 100 = 10,670 M^2 = 2.67 to 3.2 acres with other space needs. 100 trays are placed vertically, one above the other.

Maximum possible mosquito larvae and pupae population density = 37,000 per M^2 water's top surface [39].

Maximum possible midge larvae and pupae population density = 70,000 per M^2 water bottom surface [25].

Daily production of adult mosquitoes in a 7-day life cycle of larvae and pupae = 1,067,000 x 37,000 / 7 x 2 / 10^9 = 11.28 tonnes/day

Daily production of adult midges in a 21-day life cycle of larvae and pupae = 1,067,000 x 74,000 / 21 x 2 / 10^9 = 7.52 tonnes/day.

Water surface area margin available = (11.28 + 7.52) /14.97 = 1.25 (25 % margin is available in water surface area)

Enclosure building cost = 3.2 x 4000 x 10,000 x 10 = 1280 million Rs @ 10,000 Rs/M^2 and 10 floors.

Water holding tubs cost @ 1000 Rs/M^2 = 1,067,000 x 1000 = 1067 million Rs

Other plant cost @ 20,000 per M^3 /day of sewage = 20,000 x 10,000 = 200 million Rs.

Total installation cost = 1280 + 1067 +200 = 2547 million Rs.

Sale price of insect's biomass = Rs 100,000 per tonne

(1 US$ = 91 Rs)

Income from sale of insect biomass = 100,000 x 14.97 = 1,497,000 Rs/day

Annual income @ 300 days operation = 1,497,000 x 300 = 449.1 million Rs/year.

Operation and maintenance cost @ Rs 20 per M^3 = 20 x 10,000 x 300 = 60 million Rs/year

Annual gross profit = 449.1 – 60 = 389.1 million Rs.

Return on investment (equity + loan) = 389.1/2547 =15.28 %

Simple payback period = 2527 / 389.1 = 6.55 years.

Mosquitoes are responsible for the most economic damage among all invasive species [40]. This nature-based STP infrastructure can also be used to breed Wolbachia mosquitoes [41] and sterile male mosquitoes in large numbers. Wolbachia bacteria prevent viruses such as dengue, chikungunya, and Zika from replicating in Aedes aegypti mosquitoes. When released into nature in large numbers, Wolbachia mosquitoes' mate with local Aedes aegypti mosquitoes for several generations until the entire local mosquito population is infected with disease-preventing Wolbachia bacteria. Sterile Insect Technique for mosquitoes is a birth control method that involves mass-rearing radiation-sterilised males to mate with wild females without producing offspring [39].

Poultry feed production is associated with harmful greenhouse gas emissions, a large land footprint for cereals and maize/corn cultivation, and a substantial water footprint for irrigation needs [22]. Whereas farming mosquitoes and midges to produce fish or poultry feed with low land and water footprints using a BECCS process without emitting air pollutants or greenhouse gases is a superior sewage treatment method. The proposed nature-based sewage treatment method is also an economically sound proposition, generating adequate profit from its capital expenditure. This method transforms its raw material (sewage or human excreta) into a resource from a waste product that is to be safely disposed of at a cost. It would substantially contribute to the circular economy by using sewage-derived organic matter without causing pollution or greenhouse gas emissions. It is a perfect example of waste-to-wealth, extracting valuable biomass from sewage. Carbon credits/extra revenue can also be earned by selling the generated gas for local uses to replace fossil-derived CO_2 gas with carbon-neutral or bio-CO_2 gas. The treated water, being a perennial source, can be further purified for use by industries or commercial establishments as usual if found economical [42]. Each acre of land used, without a water footprint, for vertical farming of mosquitoes and midges is highly productive and can replace nearly 700 acres of irrigated land used for cereal and corn cultivation. The required land for the nature-based STP can be identified without many issues on the floodplains of streams/nallas for receiving sewage by gravity flow. The STP building can be located above the possible maximum flood level and constructed on columns/stilts to allow occasional floodwater to pass beneath the building. Existing conventional STPs can also be renovated by adding insect-rearing housings to make them economically viable and more compliant with treated-water quality standards. Wherever centralised STPs cannot be installed due to economic reasons in rural areas, sewage can be regularly vacuum-sucked by road tankers from the individual/group sewage collection sumps and transported to the nearby STP. Also, water found stagnating in open ditches, emitting a foul smell, can be vacuum-sucked by road tankers and transported to a nearby STP for treatment, since value addition to such an effort is possible by producing fish/poultry feed. It would also prevent the breeding of mosquitoes.

In 2020-21, India generated 72.37 million M^3 of urban wastewater and 39.61 million M^3 of rural wastewater [4]. Only 28% of urban wastewater undergoes some form of treatment. This indicates that 72% of untreated sewage is discharged into rivers or lakes, causing excessive eutrophication and the spread of diseases such as cholera, typhoid, and diarrhoea. As the urban population in the future reaches up to 80% of the peak

Retrieval Number:100.1/ijee.A187206010526
DOI: 10.54105/ijee.A1872.06010526
Journal Website: www.ijee.latticescipub.com

Published By:
Lattice Science Publication (LSP)

population (1.7 billion), urban sewage generation could increase by 2-3 times. It has ultimate potential to generate 77 million tonnes/year of insect biomass for use in the production of fish/poultry feed. At present, all STPs are constructed and operated by government agencies with subsidies. With the proposed STPs, the private sector can fully take over the sewage treatment business, as it is a profitable endeavour. Initially, viability gap funding can be provided by the union and state governments to encourage the private enterprises till the technology reaches a mature stage. The STP sector shall be made part of agriculture so that the income from these plants is treated as non-taxable agricultural income, encouraging participation by entrepreneurs.

IV. CONCLUSION

Mosquito species are among the most intensively researched creatures, yet they are poorly studied to derive economic benefits from their life cycles. Substantial R&D infrastructure is already available worldwide to develop methods to control breeding and propagation, preventing mosquitoes from acting as vectors in the spread of diseases to humans and animals. The existing R&D setup can be readily used for lab studies, bench plants, and pilot plants to identify suitable species, determine the optimal tolerable indoor air quality, and determine the optimal DO in sewage for rearing mosquitoes and non-biting midges to harvest insect biomass during sewage treatment.

DECLARATION STATEMENT

Some of the references cited are outdated, noted explicitly as [1], [5], [9], [15], [29], [30] and [37], However, these works remain significant for the current study, as they are pioneering in their fields.

I must verify the accuracy of the following information as the article's author.

- **Conflicts of Interest/ Competing Interests:** Based on my understanding, this article has no conflicts of interest.
- **Funding Support:** This article has not been funded by any organizations or agencies. This independence ensures that the research is conducted objectively and without external influence.
- **Ethical Approval and Consent to Participate:** The content of this article does not necessitate ethical approval or consent to participate with supporting documentation.
- **Data Access Statement and Material Availability:** The adequate resources of this article are publicly accessible.
- **Author's Contributions:** The authorship of this article is contributed solely.

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