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Process Design and Assessment of the Performance of Three Macrophytes in a Biorefinery Polishing Partly Treated Sewage in Novel SHEFROL Bioreactors

Tabassum-Abbasi ^{1,*}, Pratiksha Patnaik ² and S. A. Abbasi ² ¹ School of Engineering, University of Petroleum and Energy Studies, Dehradun 248 007, India² Centre for Pollution Control & Environmental Engineering, Pondicherry University, Puducherry 605 014, India

* Correspondence: tab.abbasi@gmail.com

Abstract: The development of a biorefinery is described based on rapid polishing of partially treated sewage with three different species of macrophytes in the recently developed SHEFROL[®] bioreactor which functions almost entirely on solar and gravitational energy, requiring no chemicals. It was seen that at a hydraulic retention time (HRT) of just 2 h, suspended solids, COD, BOD, total Kjeldahl nitrogen, soluble phosphorus, zinc, copper, nickel, and manganese are reduced to the extents of 92–95%, 76–78%, 77–81%, 56–61%, 60–66%, 43–46%, 45–48%, 30–35%, and 31–33%, respectively. The treated water meets the water quality standards set for the discharge into surface water bodies. *M. quadrifolia* was seen to be most effective of the three macrophytes, followed by *P. stratiotes* and *S. molesta*, as a sewage cleansing agent, but the difference in the performance of the macrophytes was only marginal, and not highly significant statistically ($p \geq 0.5$). The paper also shows that it is possible to extract energy precursors in the form of volatile fatty acids (VFAs) from the harvested macrophyte biomass and then convert the biomass into organic fertiliser. All the steps associated with the closed loop circular biorefinery occur at ambient temperatures and pressures, requiring little consumption of energy and materials, and leaving negligible footprints.

Keywords: biorefinery; sewage; treatment; macrophytes; *Salvinia molesta*; *Pistia stratiotes*; *Marsilea quadrifolia*



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1. Introduction

1.1. Surprising Inadequacy of Information on the Use of Macrophytes in Biorefineries

There has been increasing interest in the development of biorefineries in which wastewater treatment can be accomplished along with generation of energy and/or other resources in a manner that there is no net waste remaining to dispose. Several reports have appeared on the use of one or another species of algae as the main bioagent in such refineries, including a recent spate of reviews on this subject [1–5].

In contrast, the deployment of macrophytes in biorefineries has been to a much lesser extent so far. Only the free-floating aquatic weed pistia (*Pistia stratiotes*) has been explored in the context of a biorefinery aiming wastewater treatment [6,7]. However, the system reported by the authors—a 13,000 L lagoon filled with polluted river water—was rather slow in affecting significant treatment. It required a hydraulic retention time (HRT) of 7 days which is indicative of about 28 times slower rate of treatment than achievable by conventional wastewater treatment systems (CWTSs) based on activated sludge process (ASP) and its variants. The only other reported study referring to a macrophyte in the context of a biorefinery is also on a free-floating aquatic macrophyte duckweed (*lammnaeae*) by Calicioglu et al. [8,9], but it describes only a hypothetical biorefinery with no details on the extent of wastewater treatment achievable, or any form of experiment-based validation.

1.2. The Virtues of Macrophytes in the Biorefinery Context

The presently witnessed lack of effort in employing vascular plants in biorefineries is surprising in view of the fact that the efficacy of such plants as efficient remediators of wastewater has been well-established since over 70 years [10]. Beginning with the path-breaking work, done in the 1950s, by Kathe Sidel [11], vascular plants—both free-floating ones like water hyacinth (*Eichhornia crassipes*) and rooted ones like typha (*Typha* sp.)—have been extensively investigated as main bioagents in wastewater treatment systems [12–15]. It has also been seen that several of these vascular plants have high photosynthesis efficiency reflected in their high net primary productivity [16–18]. In fact, it is this attribute of high productivity which influences the phytoremediation efficiency of vascular aquatic plants most strongly because in order to support their rapid growth, the plants hasten the mineralization of organic carbon, nitrogen, phosphorous and other constituents of wastewaters. The nutrients that become available are utilized by the plants, thereby facilitating wastewater treatment. The activity is supported by the ability of the plants to transport oxygen via their leaves and stems to their roots [19,20]. This contributes substantially to the removal of BOD and COD.

Additionally, some macrophytes like salvinia have super-hydrophobic surfaces, which traps oxygen [21], aerating the water and helping the degradation of organic carbon.

In addition to the attributes mentioned above, a great deal of information is also available on the utilization of vascular plants, after completing their role in wastewater treatment. Among numerous substances that can be obtained from vascular plants are (a) biogas, (b) biohydrogen, (c) biodiesel, (d) drugs and other chemicals (e) paper pulp; (f) fertilisers, (g) nanoparticles [22,23].

It is noteworthy that many vascular plants have higher growth rates and net primary productivities than are achievable by algae [16]. They are also more resilient and robust. Most importantly, use of vascular plants in wastewater treatment does not necessarily require energy and material inputs in the form of agitating and filtering devices which are unavoidable in algae-based systems.

1.3. Emerging Role of the SHEFROL Technology

The only drawback that has prevented vascular plants, especially aquatics, from being widely used in wastewater treatment has been the lower efficiency, as compared to CWTSSs, of the systems based on these plants [24]. The lower efficiency translates to larger hydraulic retention times (HRTs) needed for the wastewater to reach the desired level of treatment. This, in turn, increases the land area requirement which often makes the systems unviable due to rising land costs.

However, this major shortcoming has been recently overcome by these authors with the introduction of a novel bioreactor. It has been acronymed SHEFROL to reflect its 'sheet flow root level' throughput of wastewater. It is a novel system which has received a patent [25] as well as a trademark [26].

Extensive trials, first at bench-scale and subsequently on several pilot plants of capacities ranging from 20,000 L per day (LPD) to 120,000 LPD, have established the viability, versatility, robustness, efficiency, and efficacy of SHEFROL bioreactor [24,27,28]. The running of pilot-scale field trials continuously for several years has also established the ability of the reactor in handling wide variations in influent volumes as well as characteristics. Additionally, the reactor's ability to handle shock-loads has been confirmed time and again.

Above all, the trials have established that even as SHEFROL reactors are as efficient as the CWTSSs, they are several times less expensive, with negligible footprints [24].

1.4. The Present Work

In the course of working on SHEFROL[®] systems, the present authors have come across several situations where existing CSTSs based on ASP and its variants were unable to bring the concentrations of COD, BOD, and other pollutants in their effluents to the levels that can meet the statutory discharge standards. Such situations had arisen because of the

practice often encountered in the developing countries wherein blackwater and/or eatery wastewater is discharged into drains meant for carrying only greywater to the CSTSs. The consequent overload on the CSTSs adversely effects their designed performance level, resulting in the treated sewage having inadequate quality.

In view of the high rate of treatment achievable with SHEFROL[®] reactors at very low costs it was considered worthwhile to explore whether SHEFROL[®] reactors can be used at very low HRTs, in other words at much faster rates of wastewater throughput (hence negligible cost), for polishing partly treated sewage to the extent that it can meet the statutory reuse/discharge standards. Accordingly, the present work was undertaken. It has also been aimed to assess the relative efficacy of three macrophytes as bioagents for the purpose. These include two free-floating aquatic weeds: salvinia (*Salvinia molesta*, Mitchell), and pistia (*Pistia stratiotes*), and the four-leaf-clover (*Marsilea quadrifolia*), which is a marsh plant that exists rooted to the moist soil in nature.

2. Materials and Method

2.1. Approach to Process Design

Given that the objective was to achieve desired level of polishing of sewage in the shortest possible time and at the minimum essential cost, it was decided to study the refinement of the partially treated sewage by SHEFROL at different HRTs. SHEFROL[®] technology is based on bioreactor comprised of narrow channels of which the dimensions have been optimized. In these channels, adult but short-statured macrophytes are stocked to capacity and wastewater is made to flow through the channels as a sheet thick enough to cover the roots of the macrophytes. The acronym SHEFROL comes from this sheet-flow-root-level hydrology adapted in SHEFROL technology to maximize aeration and contaminant removal at minimum inputs of energy and materials. No scaffold or anchor is required/provided to hold the macrophytes, nor are any pumping devices, agitators, or aerators needed. The reactor runs on direct solar and gravitational energy. The solar energy supports growth of the macrophyte stocked in the reactor channel, driving wastewater treatment in the process. Gravitational energy is deployed in controlling the wastewater flow-rate (in turn HRT) on the basis of difference in the liquid head at the point of the entry of the wastewater into the SHEFROL channel and at the point of the exit of the treated wastewater from it. The system is green and clean (Figures 1 and 2).



Figure 1. A typical SHEFROL plant treating sewage with duckweed as the main bioagent.



Figure 2. Another SHEFROL plant treating sewage continuously. It is planted with *Salvinia molesta*.

Study of the effect of different HRTs on treatment was aimed to identify the lowest HRT which was adequate for the purpose of polishing the sewage to desired levels. It may be clarified that HRT is defined as $\frac{V}{q}$, where V is the reactor volume (m^3) and q is the influent flow rate (m^3/hours). The more efficient a reactor in terms of speed of its operation, the higher q it can maintain for a given level of performance. Proportionately lesser is the reactor volume needed. Considering that upto 80% cost of all processes is due to their reactors, HRT becomes the main driver of process cost. Three different species of macrophytes were explored to see how sensitive the process is to the nature of bioagents.

2.2. Reactor Fabrication and Process Monitoring

Accordingly, bench-scale SHEFROL[®] reactors were fabricated and operated. This was done essentially with the use of similar material and similar manufacturing steps as detailed earlier [19,29,30]. The schematic of the resulting system is given in Figure 3. In three sets of duplicate SHEFROL[®] channels, *P. stratiotes*, *S. molesta*, and *M. quadrifolia*, respectively, were propagated. The ‘seed’ plants for this purpose were obtained from ponds and marshes situated near the author’s place of work. Two channels, which were kept free of any plant, served as a control.

The reactor was run at HRTs of 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 h. With the channel volume being constant, the HRT was varied by varying the influent flow rate. This, in turn, was achieved by appropriately adjusting the influent liquid head at the entry point.

As per the United States Environmental Protection Agency [31], on-site domestic wastewater treatment plants must deliver effluents with BOD, suspended solids, and ammoniacal nitrogen not exceeding 20, 30, and 20 mg/L, respectively. A World Health Organization [32] compendium on standards for discharge of treated sewage by different countries in the Eastern Mediterranean region shows that the limit for BOD, set by different countries, varies in the range 20–60 mg/L. The ranges for total nitrogen (N) and suspended solids (SS) encompass 60–70 and 30–60 mg/L. The Indian standards, summarized in Table 1, indicate that the strictest of standards are for discharge into inland surface water bodies. These stipulate 30 mg/L as the limit of permissible BOD in wastewater for discharge. This limit is close to, or within, the stipulations of different countries quoted above, but,

surprisingly the Indian standards put the maximum permissible COD, for the same purpose, to be as high as 250 mg/L. Considering that the concentration of BOD in sewage is generally 40–60% of COD, in other words the COD:BOD ratio lies in the 1.7–2.5 range, these standards—which accommodate effluents having 8.3 times more COD than BOD—are highly unusual. This kind of COD:BOD ratio can occur only in industrial effluents carrying large concentrations of non-biodegradable organics and is unlikely for sewage. Hence for the purpose of monitoring the performance of SHEFROL in the present study, it was decided to assess its ability to meet the limits prescribed in Table 1 for discharge into inland surface water bodies, putting the upper limit for COD to be 45 mg/L (i.e., 1.5 times the maximum permissible BOD), instead of going by the limit of 250 mg/L for COD set in the said standard. Considering that the other three standards (Table 1) are more liberal, if the SHEFROL effluent can meet the most conservative of the standards it will be more than adequate for the rest as well.

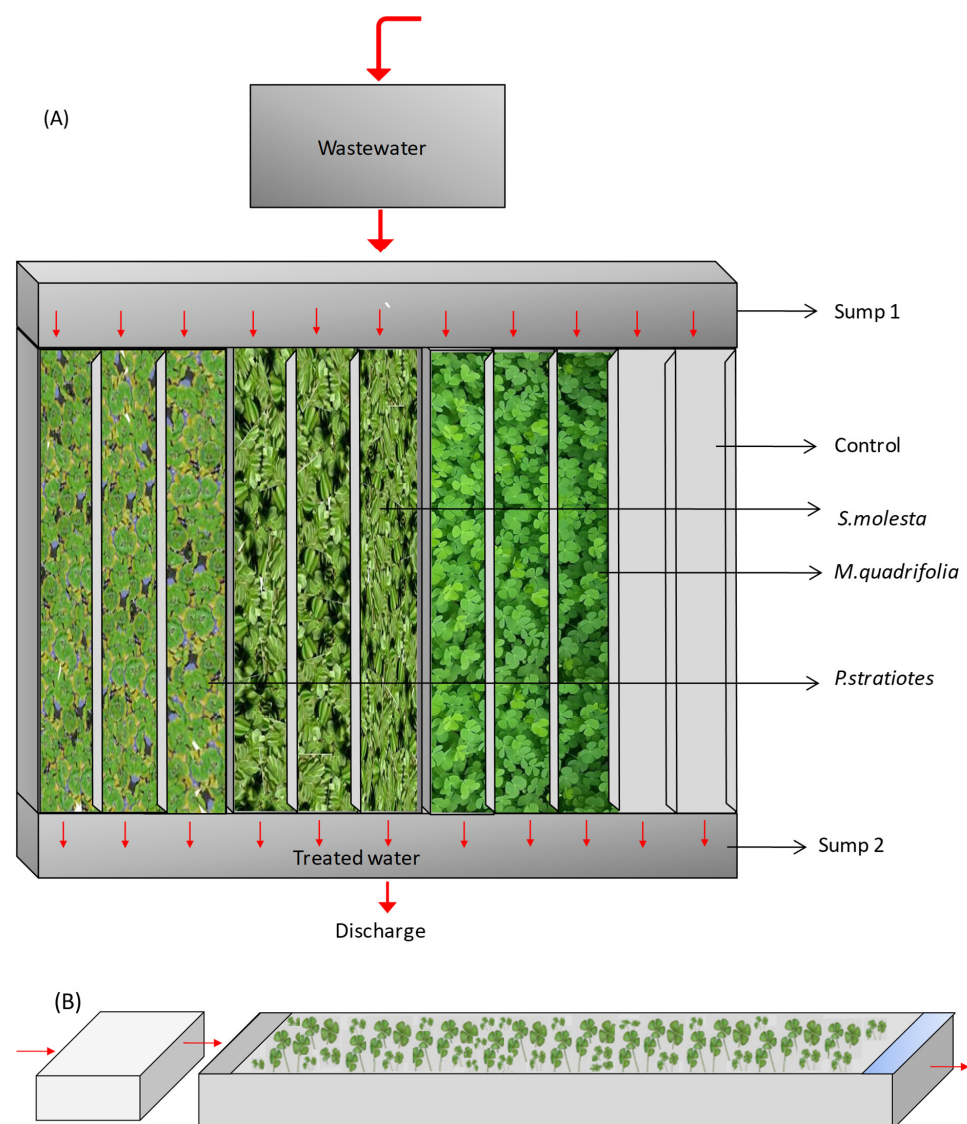


Figure 3. Schematic of SHEFROL, top view (A) and side view (B).

Table 1. Indian standards for the discharge of wastewater [33].

Parameter	Standards for Discharge			
	On Inland Surface Waters	In Public Sewers	On Land for Irrigation	In Marine Coastal Areas
SS, mg/L	≤100	≤600	≤200	≤100
pH	5.5–9.0	5.5–9.0	5.5–9.0	5.5–9.0
TKN, mg/L	≤100	–	–	≤100
BOD, mg/L	≤30	≤350	≤100	≤100
COD, mg/L	≤250	–	–	≤250
Cu, mg/L	≤3.0	≤3.0	–	≤3.0
Zn, mg/L	≤5.0	≤15	–	≤15
Ni, mg/L	≤3.0	≤3.0	–	≤5.0
Mn, mg/L	≤2.0	≤2.0	–	≤2.0

2.3. Sampling and Analysis

In order to monitor the characteristics of the sewage as it entered SHEFROL[®] channels, and the extent to which the characteristics were improved in the polished wastewater exiting the reactor, samples were drawn from the points of the influent's entry to, and exit from, the SHEFROL[®] channels. The drawing of the samples, their preservation, and analysis was done as per standard methods set by the American Public Health Association [34]. Three sets of the influent and effluent samples were drawn daily at 14 h, and separately pooled before they were analyzed immediately after the sampling. Biochemical oxygen demand (BOD), suspended solids (SS), and total Kjeldahl nitrogen (TKN) were determined by wet chemical methods. Soluble phosphorus (SP) was estimated spectrophotometrically with the help of a Lab India—UV 3000+ machine (Matrix Technochem, Thane, India). For heavy metals, a Perkin Elmer AA 800 atomic absorption spectrometer (Perkin Elmer, Inc., Waltham, MA, US) was deployed. It was supported by its flameless atomizer accessory.

The precision of the analysis of all variables was ensured by checking the reproducibility of each estimation while the accuracy was achieved by assessing recovery using the method of standard addition. The latter was done to ensure that matrix interferences do not affect the analysis results [34–36].

2.4. Use of COD as an Indicator Parameter and Analytical Quality Control

To enable monitoring of the reactor performance frequently, quickly, and inexpensively, chemical oxygen demand (COD) was used as an indicator or 'tracer' parameter. This was done for four main reasons: (a) COD includes biological oxygen demand (BOD) and hence reflects the action of the macrophytes on most forms of organic carbon present in the wastewater; (b) it can be rapidly assessed in contrast to BOD which takes 5 days; (c) biodegradable carbon is the largest component that requires treatment in greywater; and (d) plants always take up nitrogen, phosphorus, and other elements from water for their growth; hence, those components of greywater are certain to be reduced if a macrophyte can survive in the sewage and reduce its organic carbon. Several past studies [19,30] have validated this reasoning.

2.5. Statistical Analysis

Assessment of the extent of statistical significance in the differences in the macrophyte performance and between the treatment occurring in macrophyte-planted channels in comparison to control channels was assessed by appropriate 't' tests [37]. The software Microsoft Excel was deployed for the analysis, and it was tested whether the 't' test supports or rejects the null hypothesis of there being no difference between the sets of results being tested. The difference was accepted as significant if it occurred at confidence levels $\geq 95\%$ ($p \leq 0.05$).

3. Results and Discussion

3.1. Polishing of Sewage Achieved by the Three Macrophytes

The characteristics of the partially treated sewage, obtained on the basis of sampling and analysis performed thrice a day at 8, 14, and 18 h for a week, was found to vary in the range shown in Table 2. Variations of these extents are expected because of the differences in the quantities of water that are used at different times of the day, and the purpose for which they are used. Similarly, drastic variations in sewage characteristics have been recorded by these authors not only from hour to hour, but season to season as well [24,28]. Moreover, all desirable sewage treatment systems are expected to accommodate such variations, even more strong shock-loads, without adversely affecting the extent of treatment. SHEFROL[®] has been seen to fulfill this requirement [24,28].

Table 2. Characteristics of the partly treated sewage which was fed to SHEFROL.

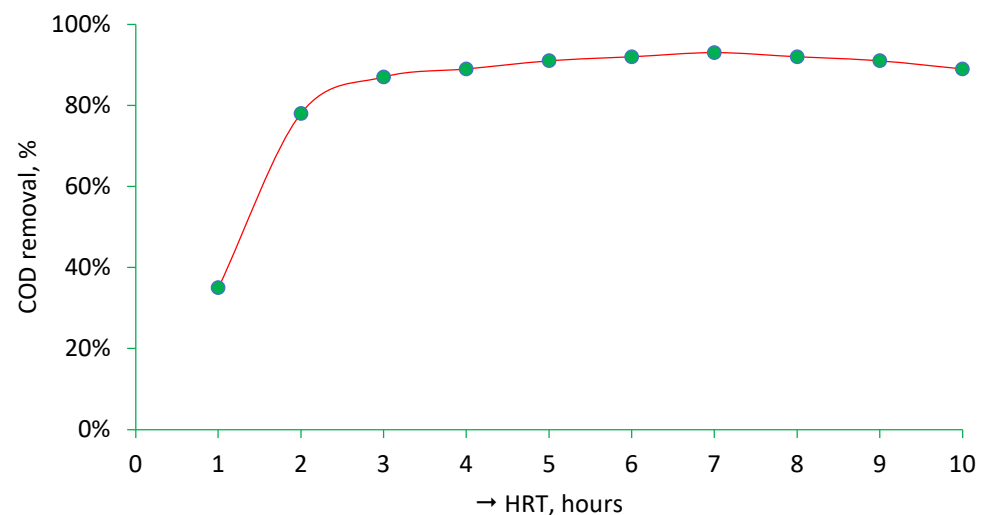
Characteristic	Range, mg/L
BOD	30–60
COD	55–90
SS	60–85
TKN	37–55
SP	2–4
Zn	2–6
Cu	1–4
Ni	2–4
Mn	1–2

At each HRT, significant treatment of the influent (partly treated sewage) was achieved from the very first day (24 h from start of the reactor), as reflected in COD removal of $15 \pm 3\%$. The extent of treatment kept improving with every passing day in all channels till a stage came, within 5–8 days of the start, when the treatment level reached a peak and then began hovering around a mean. It signaled the attainment of a steady state. All figures of treatment discussed below correspond to the respective steady state which, for that HRT and macrophyte, indicates the maximum treatment achievable.

The performance of the three macrophytes, in terms of the removal of the indicator constituent COD from the influent, is summarized in Table 3. The pattern has been illustrated with the example of *M. quadrifolia* in Figure 4. It is seen that the most rapid improvement in COD removal occurs as the HRT is increased from 0 to 2 h. Even more specifically, the most drastic improvement in the treatment occurs between HRT 1 and 2 h. The curve then begins to plateau, with only minor improvement in the treatment as the HRT is increased to 7 h. Even at higher HRTs, the level of treatment does not improve further. Instead, it begins to fall. This may be happening due to recontamination of sewage by plant debris when the rate of flow-through falls below a threshold with the rise in the HRT. The pattern of the variation of the treatment level with the HRT was similar in case of the other two macrophyte species; the performance of *P. stratiotes* being next best, followed by *S. molesta*. However, the difference between the extents of treatment achieved by the three was not highly significant statistically ($p \geq 0.5$). In the control channels, removal of COD, BOD, and SS was always below 8%, and the removal of other variables was always below detection limits. The marginal reduction in COD and BOD in the control channels was evidently due to the action of sunlight and aeration on the passing sewage while the removal of SS was perhaps due to settling of some of the coarser solids. In all cases, the difference in the level of treatment between any of the macrophyte-planted channel and either of the control channels was highly significant ($p \leq 0.1$).

Table 3. Average COD removal at steady state in SHEFROL at different HRTs by the three macrophytes.

HRT, Hours	Average Steady State COD, %, Removal by		
	<i>P. stratiotes</i>	<i>S. molesta</i>	<i>M. quadrifolia</i>
1	34 ± 2	32 ± 2	35 ± 2
2	77 ± 3	76 ± 2	78 ± 2
3	85 ± 3	84 ± 3	87 ± 2
4	88 ± 2	86 ± 3	89 ± 2
5	89 ± 3	88 ± 3	91 ± 3
6	91 ± 3	90 ± 3	93 ± 2
7	92 ± 3	90 ± 2	93 ± 3
8	91 ± 3	89 ± 3	92 ± 3
9	90 ± 3	90 ± 3	91 ± 2
10	88 ± 2	87 ± 3	89 ± 3

**Figure 4.** Average COD removal at different HRTs in SHEFROL channels stocked with *M. quadrifolia*, after steady state had been reached.

The results indicate that an HRT of 2 h is optimal for the use of SHEFROL[®] in polishing of partly treated sewage because at higher HRTs the improvement is not proportionate to the process time consumed. Given that the lesser the HRT, lesser the reactor size, consequently, lesser is the cost of treatment per litre of the wastewater, if there is an increase in the HRT beyond 2 h, it is not seen to bring benefits proportional to the likely increase in the reactor costs.

The average levels of treatment of other constituents attained at 2 h HRT are given in Table 4. The maximum levels recorded of each constituent in the polished sewage are also shown in Table 4. They are all below the most conservative limit set by the concerned standards (Table 1), indicating that the polishing achieved in SHEFROL by all the three macrophytes at 2 h HRT was adequate to meet all the discharge standards (Table 1).

It needs to be emphasized that in different regions, there may be differences in the characteristics between varieties of the same species. Even within the same location, the extent of effectiveness of a macrophyte in cleansing sewage may differ from season to season. Matrix effects (presence of, and concentrations of, other pollutants) may also play a role. Hence the results of this study are indicative of the general pattern, and actual figures may differ slightly separate from season to season and location to location. The results do show with certainty that SHEFROL[®] can be used very effectively in polishing partly treated sewage to certain levels so that the effluent meets all discharge standards, and can also be used in irrigation. The studies also show that very widely and freely available weeds

like *salvinia*, *pistia*, and *marselia* can be used to very inexpensively achieve the required polishing, with negligible ecological footprint, by the SHEFROL[®] technology.

Table 4. Average removal of different constituents at of each constituent steady state by the three macrophytes at the HRT of 2 h, and the maximum concentration recorded in the effluent.

Constituent	Average Removal, %, by			Maximum Observed Concentration in the Effluent, mg/L
	<i>P. stratiotes</i>	<i>S. molesta</i>	<i>M. quadrifolia</i>	
BOD	79 ± 3	77 ± 3	81 ± 3	15.6
COD	77 ± 3	76 ± 2	78 ± 3	23.4
SS	94 ± 3	92 ± 3	95 ± 3	9.4
TKN	59 ± 2	56 ± 2	61 ± 3	25.3
SP	60 ± 3	63 ± 2	66 ± 3	1.7
Zn	43 ± 4	43 ± 3	46 ± 3	3.7
Cu	46 ± 2	45 ± 2	48 ± 2	2.3
Ni	32 ± 3	30 ± 1	35 ± 2	2.8
Mn	31 ± 2	31 ± 2	33 ± 3	1.4

3.2. Mechanism of Pollutant Removal

From the time the first-ever scientific study was conducted on the use of macrophytes in the phytoremediation of sewage by Kathe Sidel [11], attempts have been made across the world to understand the mechanism associated with it. The initial expectation was that macrophytes may be absorbing or taking up the pollutants, though it was soon dispelled when studies revealed that macrophyte uptake has only a limited contribution to the contaminant removal [16]. Further experiments led to the realization that it is the microflora present in and around the roots of the macrophyte—its rhizosphere—and which is more than 100 times as diverse as the microflora in the rest of the plant/wastewater that exerts a decisive influence. By-and-by, the role of other factors, such as the biopolymers and enzymes exuded by the roots, the aeration of the rhizosphere caused by the transport of atmospheric air to the rhizosphere via the macrophyte leaves and stem, and the ‘salvinia effect’, have also come to light [13,14,19,38–40]. Due to the combined effect of all of these forces, there is rapid decay of BOD and COD as the wastewater passes through the macrophyte roots. The biopolymers that are secreted by the roots cause flocculation and consequent removal by settling of the SS. A number of physicochemical and biochemical factors facilitate the removal of N, SP, and metals. How these and other factors help the macrophytes in facilitating contaminant removal from sewage have been discussed by these authors [19,24], with specific reference to the attributes of SHEFROL[®] which maximize the gains from the contaminant removal mechanism. Most notably, (a) the design and operation maximizes the contact of the passing wastewater with the plant roots, thereby deriving maximum benefit from the microflora and biomolecules present in the rhizosphere; (b) the design and operation also maximise air (oxygen) transfer from the atmosphere to the wastewater, both directly and through the plant leaves, thus removing one of the most serious bottlenecks of the constructed wetland-based systems. It can be safely assumed that all these factors are operative in the rapid polishing of sewage reported here.

4. Generation of Energy Precursors and Organic Fertilisers from the Harvested Macrophytes to Close the Biorefinery Loop

All the three species tended to get rapidly established in the reactor channels and begin to grow vigorously. Indeed, their propensity to utilize the nutrients available in the sewage for their growth, supported by their high primary productivity, are the main driving forces that cause treatment of sewage. The generally high resilience and growth rate, which has made these species weedy, is seen to become a virtue in the context of sewage treatment. The continued survival and growth of the macrophytes in SHEFROL[®] enabled the reactor to be self-propagating, needing no energy input other than direct solar radiation. The only maintenance requirement was to harvest the extra growth—which

was in the range 2.5–4 kg fresh weight per month—once in 2–3 weeks besides removal of dead plants. By putting this extracted biomass to use in such a way that no waste is left to dispose, the resulting system can be upgraded to a closed loop biorefinery.

Hence, after ascertaining the feasibility of wastewater polishing the biomass production in the SHEFROL[®] systems, as well as the feasibility of all the three macrophytes that were explored for this purpose, the next step was to explore the options for utilizing the 'spent' and the overgrown macrophytes in a way that useful products are generated but no net waste is created.

As mentioned earlier in Section 1.2, several studies have been reported on generating a variety of products from vascular plants ranging from medicinals, cosmetics, other chemicals, paper pulp, food/ feed, and nanoparticles, just as similar studies have been reported on algae as well. Despite very extensive research conducted world-wide on this aspect however, no large-scale applications of any of the options have been possible, much less for any macrophyte or any algal species. This is due, basically, to the fact that in nearly all the cases, there are more efficient and less expensive options available for generating the same products from other sources/processes. For instance, efforts to utilize macrophytes/algae as feedstock in anaerobic digesters for generating bioenergy in the form of 'biogas', pose special challenges that are not encountered when animal manure is the feedstock, or when wastewaters are subjected to anaerobic digestion [13,41]. Moreover, neither biohydrogen, nor solid/liquid biofuels have been obtained from macrophytes/algae in a manner that is economically viable. Furthermore, final disposal of the biomass, after energy or other products have been obtained from it, has remained a challenge [42].

These authors have tried to remove both the constraints. It has led to the following:

(a) Development of methods with which energy precursors can be extracted from phytomass in the form of aqueous solutions of volatile fatty acids, or VFAs [43,44]. The VFA solutions can then be fed to any anaerobic digester, ranging from a low-rate batch-fed system to a high-rate continuous system, to obtain energy in the form of biogas.

(b) Conversion of the biomass, remaining after the VFA extraction, into organic fertilizer, invoking the novel high-rate vermicomposting paradigm, and the technology developed on its basis [45,46]. The technology enables direct and rapid conversion into high quality organic fertilizer of botanical species, such as *Salvinia molesta* [47] and *Azadiracta indica* [48]. The process occurs at rates 3–4 times faster than possible with conventional vermicomposting systems. Equally importantly, such rapid vermicomposting is achieved without the need of any pre-treatment or manure/enzyme/nutrient supplementation, thereby improving the process economics. Further, the process makes it possible to convert even toxic and allelopathic weeds like *Prosopis juliflora*, *Ipomoea carnia*, *Lantana camera*, and *Parthenium hysterophorus* to benign organic fertilizers [49–53]. Hence by attaching a vermicomposting step downstream SHEFROL[®] units, the system can be transformed into a biorefinery in which partly treated sewage can be polished using one or other of the three macrophytes, and the spent/grown biomass of the macrophytes can be converted into organic fertilizer, leaving no net waste to dispose.

Both of the processes are operable under conditions of ambient temperatures and pressures, requiring very little inputs of energy and materials. Whereas the use of biogas as fuel is already well-established, the virtues of phytomass-based vermicomposts as organic fertilizers have been extensively tested and validated by the authors [54]. It has been shown that even macrophytes, such as salvinia and ipomoea, which possess toxicity as well as allelopathy, get transformed into excellent fertilizers which are as effective in or even better at promoting plant growth, fruit yield, and fruit quality than other organic or chemical fertilizers [55–57].

Hence by exercising these options, the sewage-polishing SHEFROL[®] system can be transformed into a circular and closed-loop biorefinery, which (a) treats wastewater while generating biomass (concomitantly sequestering carbon); (b) yields energy precursors (c) generates organic fertilizer, and (d) leaves no net waste to dispose.

The present work also brings out the ease of use and the effectiveness of macrophytes in biorefineries and makes a case for further work in this area.

5. Summary and Conclusions

The effectiveness of three macrophytes, which included the free-floating aquatic weeds salvinia (*Salvinia molesta*, Mitchell), pistia (*Pistia stratiotes*), and the marsh plant four-leaf-clover (*Marseliaquadrifolia*), was evaluated in the rapid polishing of partly treated sewage in SHEFROL[®] bioreactors. It was seen that at a hydraulic retention time (HRT) of just 2 h, which represents a rate at least 3 times faster than conventional sewage treatment technologies, there was substantial reduction in suspended solids, COD, BOD, total Kjeldahl nitrogen, soluble phosphorus, zinc, copper, nickel, and manganese—to the extents of 92–95%, 76–78%, 77–81%, 56–61%, 60–66%, 43–46%, 45–48%, 30–35%, and 31–33%, respectively. The water was treated to the levels that met the most stringent of water quality standards set for discharge of treated sewage. Of the three species tested, *M. quadrifolia* was a shade more effective than the other two species, while *P. stratiotes* was marginally better than *S. molesta*. All three of the species were capable of adequately polishing the partly treated sewage to the required extent. The overall system has negligible footprints because it relies almost entirely on direct solar and gravitational energies. The feasibility of upgrading this system into a biorefinery has also been discussed on the basis of integrating it with downstream steps of extraction of energy precursors in the form of volatile fatty acids (VFAs), and the final and total utilization of biomass by converting it into an organic fertilizer. The findings provide fresh evidence of the efficacy and versatility of the SHEFROL[®] technology as a sewage treatment process which can be easily upgraded into a closed-loop, zero waste biorefinery.

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