Resort Wastewater Treatment System using BioGill Technology

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Abstract

A BioGill wastewater treatment system was retrofitted into a rudimentary sewerage network on an Island resort in Fiji to treat the relatively strong and refractory wastewater to a standard suitable for reuse via sub-surface irrigation. The system had a 6.0 m^3 primary settling tank and a 26 m^3 aerobic treatment tank fitted with two BioGill cores with a total of 640 m^2 of gill membrane area and a hydraulic recirculation rate through the gills of 12 m^3/hour. Samples were tested in a field laboratory on-site and in the National Water Quality Laboratory in Suva on the mainland, which took two days to reach. The resort had peak occupancy up to a total of 100 persons including staff whom also lived at the resort. The influent was relatively rich and was fed water from the commercial kitchen rich in fats, oils and grease, leachate from a total of 12 Clivus-Multrum composting toilets with biochemical oxygen demand (BOD) of 5666 mg/L and total Kjeldahl nitrogen (TKN) of approximately 25,700 mg/L, shower and laundry water averaging in total 13.3 m^3/day. The influent BOD averaged 662 mg/L, the TKN averaged 112 mg/L and the total phosphorous (TP) was 3.9 mg/L. The primary treatment system remarkably reduced the wastewater BOD to an average of 320 mg/L with an average hydraulic retention time (HRT) of 14 hours. This was most likely due to the formation of ammonium soaps that formed a thick scum layer on the surface. The BioGill system treated the settled influent to produce a final effluent with an average BOD of 25 mg/L, total suspended solids of 9.6 mg/L, TKN 21 mg/L and TP 4.0 mg/L with an average HRT of 15 hours (average total treatment time of 29 hours). Approximately 81% of the TKN entering the system was removed, which was due to efficient nitrification and concurrent denitrification, which was attributed to the repeated oxic anoxic cycling in the system and the possibility that autotrophic ammonia oxidation, heterotrophic ammonia oxidation, fungal ammonia oxidation and aerobic denitrification took place in the gills and anaerobic denitrification occurred in the decant tank. No biological phosphorous removal was detected, which was due to the separation of the suspended biomass from the aerobic gills. Water exiting the resort as groundwater was tested for total organic nitrogen and nitrate concentrations, which averaged 0.57 mg/L and 1.60 mg/L, respectively. The irrigation water was attributed as being partly responsible for the rapid growth of the gardens and the low nitrogen levels in the groundwater entering the reef was attributed as the major cause of the recession of the algal bloom at the low-tide level and the regeneration of the reef seen during the eight month period since the commissioning of the BioGill treatment system.

Introduction

A Bio-Gill aerobic wastewater treatment system was retrofitted onto an existing sewage treatment system at an Island Resort in the Yusawa Islands Fiji in November. With the objective being to improve their effluent quality to a standard suitable for sub-surface irrigation to irrigate the resort's gardens and to reduce the nutrient load discharged to the ocean, which was adversely affecting the local coral reef ecosystem by promoting an algal bloom (Figure 1) in a 10-15 m wide zone extending out from the low tide line.

The commissioning was cut short with the system operating, but no test data was recorded on the systems performance. For validation and warrantee purposes samples from the system were analysed in the field and by an independent laboratory in Suva to certify that the systems performance was compliant with Fijian regulations regarding wastewater treatment and environmental discharge. Another aim of the project was to test groundwater exiting the resort into the environmentally sensitive reef surrounding it to confirm compliance with environmental regulations.

The BioGill technology has until now been subject to a degree of secrecy due to the patent process, so has only been described in limited detail in conference proceedings. The basic features of the technology are depicted in Figures 2-4. The metabolic processes and microorganisms that grow on the gills have not been characterised to the degree of detail that has been performed for conventional technologies, so the processes have been inferred and deduced from seven years of research [1-6].

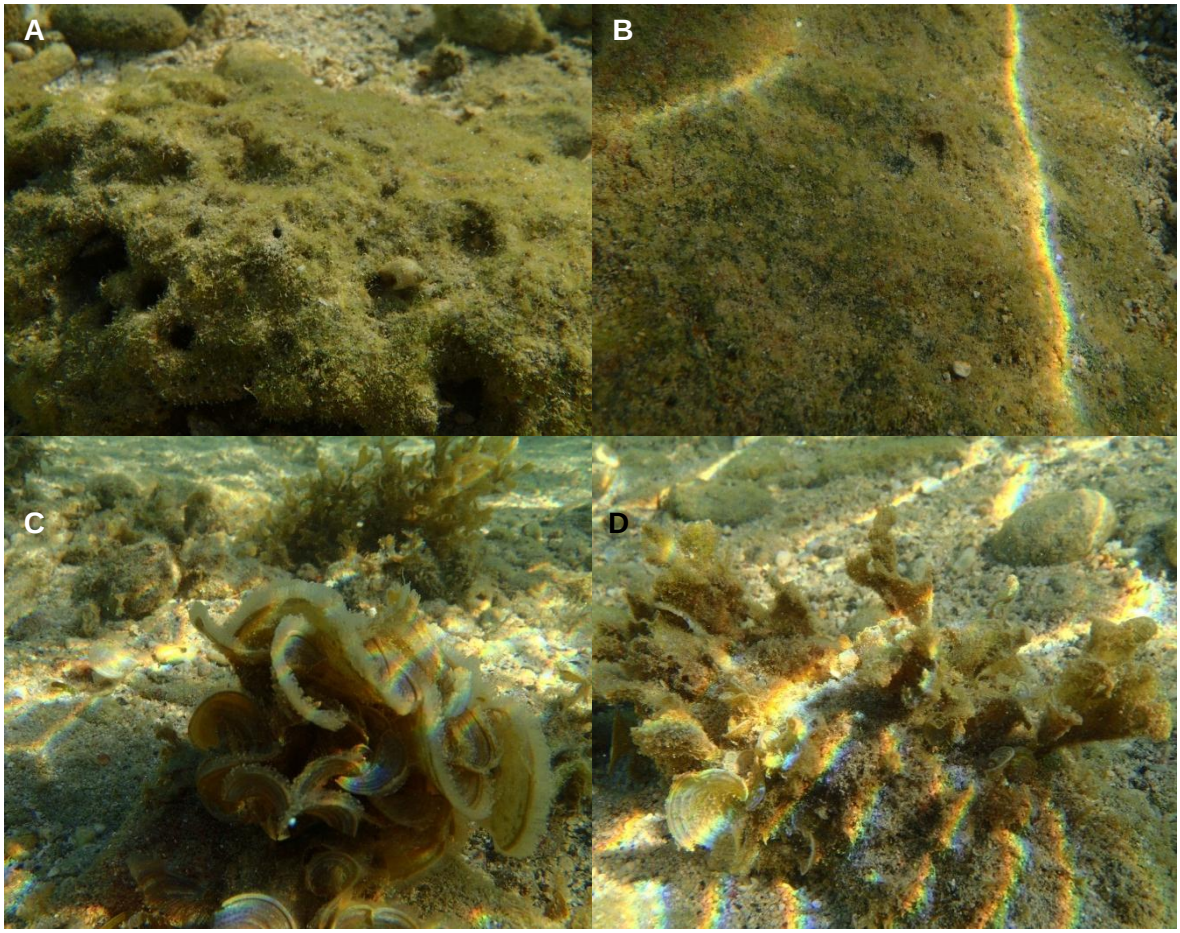

Figure 1: A) Microalgae smothering coral near the shore-line at the resort in the channel; **B)** more microalgae smothering coral near the shoreline; **C)** and **D)** further away from the shoreline macro algae flourished.

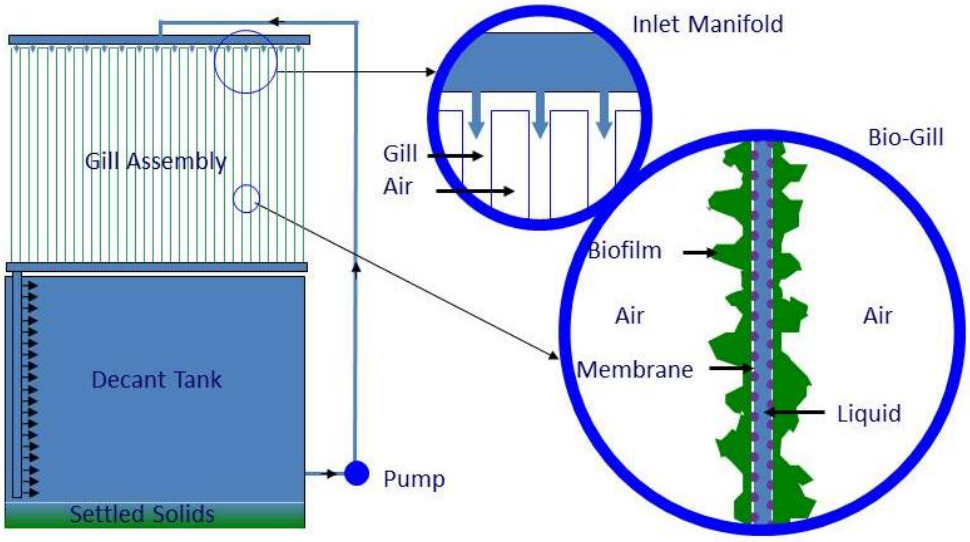

Figure 2: Schematic diagram of the BioGill treatment technology involving wastewater in a decant tank being recirculated through a set of gills via a pump, returning to the tank under gravity via an energy disperser. Each gill consist of a pair of planar membranes oriented vertically with the liquid trickling down between them and with air surrounding the gills to feed oxygen to the biomass that grows on the membranes from one side and soluble nutrients from the other side. This structure is fundamentally different to all other wastewater treatment architectures which rely on submerged cultures, where both the nutrients and oxygen are both supplied in the liquid from one direction.

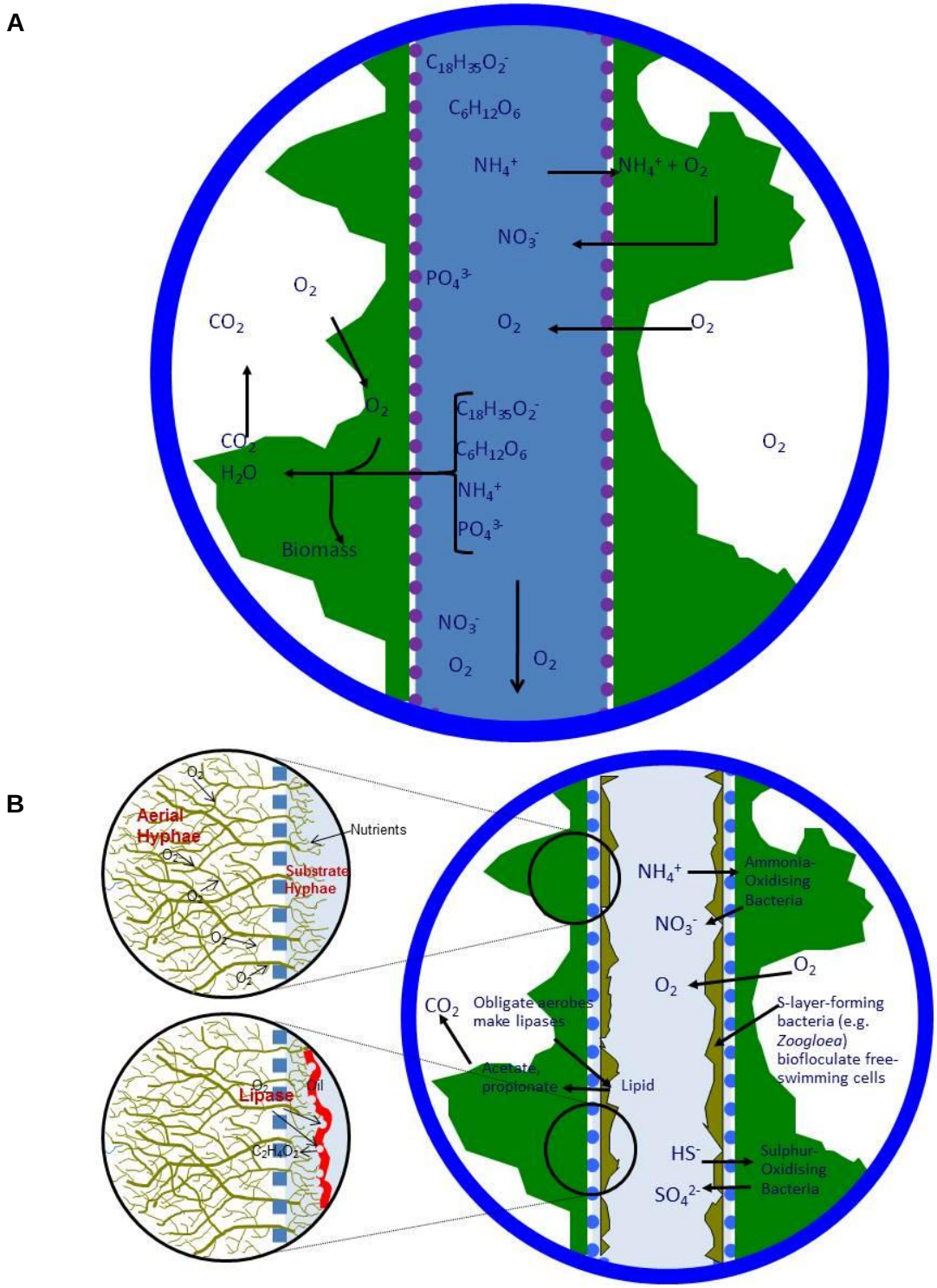

Figure 3: A) Schematic diagram of the biochemistry that hypothetically takes place in the BioGill, depicting catabolic oxidation of organic compounds such as sugars and free fatty acids and ammonia oxidation and nitrification to feed anabolism. The elevated availability of oxygen promotes the production of CO_2, which in turn feeds autotrophic nitrification; and **B)** a schematic diagram of the biochemistry that hypothetically takes place in the BioGill, depicting ammonia oxidation, sulphide oxidation to sulphate, degradation of lipids via lipase enzymes produced by obligate aerobic fungi producing acetate and propionate, and predation of free-swimming bacteria by S-layer forming microbes such as *Zoogloea* sp. growing on the inner face of the membranes. Note that fungal biomass growing as a dry surface culture has a drastically elevated surface area relative to the surface area of the membrane, which promotes oxygen mass-transfer for elevated metabolic activity so the metabolisms of the biofilm inhabitants are only limited by nutrient concentration not oxygen availability, as is the case in submerged cultures. Nitrate and sulphate act as alternate electron acceptors for anaerobic metabolism in the decant tank by denitrifying bacteria and sulphate reducing bacteria. When the reduced nutrient concentrations become low

enough the gills become an efficient oxygen-transfer device, which makes the water in the decant tank approach saturation to promote the settling of solids.

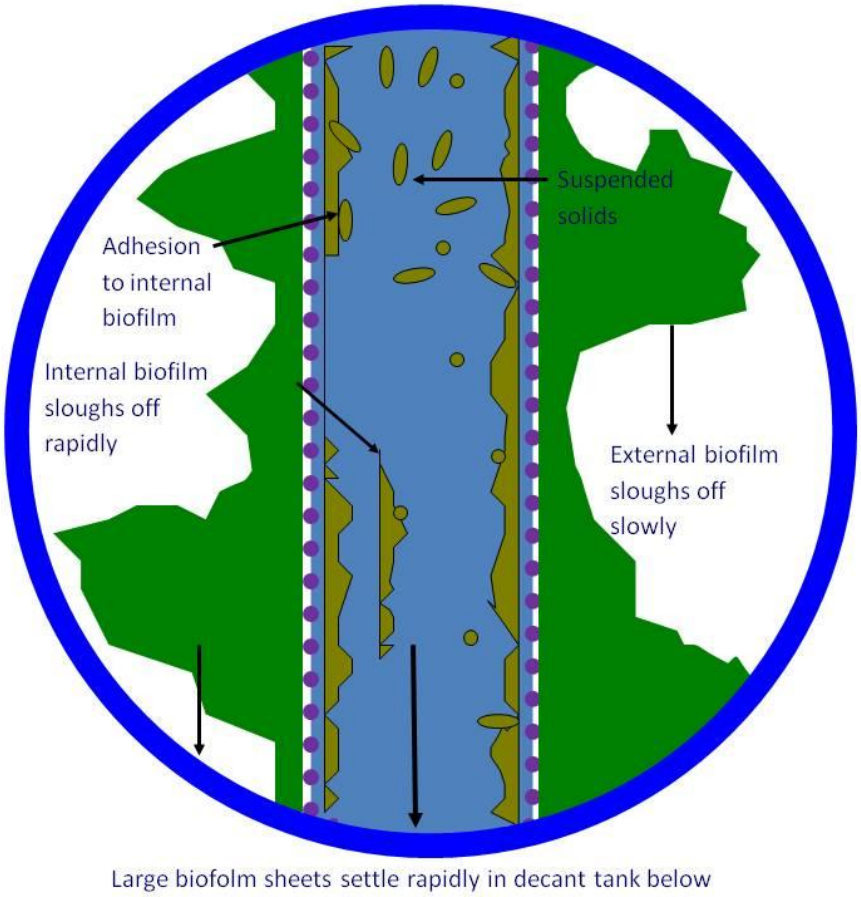

Figure 4: Schematic diagram of the biophysics of the gills, which hypothetically bioflocculate suspended solids and free-swimming cells via S-layer producing bacteria growing on the inside of the membranes. The biofilms then slough off and settle rapidly as large pieces in the decant tank below. Note that aeration and settling take place concurrently within the system, not sequentially as occurs in conventional technologies such as activated sludge. This enables very low suspended solids concentrations to be achieved without the need for filtration.

The fundamental difference between the BioGill technology and all other wastewater treatment technologies is that the biomass grows as biofilms in direct contact with the air and are fed soluble nutrients via a porous silica-polyethylene terephthalate membrane, which prevents the biomass from being smothered by water and/or oil, so the availability of oxygen to the biomass is always very high. This enables very high lipase activity by obligate aerobic fungi and bacteria to degrade the oils without the biomass being smothered as happens in other technologies. The gills act as an ideal habitat for various bacteria and fungi and prevents biomass washout, regardless of the flow rate. Old biomass sloughs off the membranes and is replaced with new cells, so the system is constantly refreshing itself and selecting for active biomass.

Rapid growth rates have been recorded due to the abundance of oxygen [7]. Fungal biomass growing on the dry outer surfaces of the gills produce aerial hyphae that gives the biofilm a very high surface area with an estimated 2000-4000 m^2/m^2 of membrane when rich wastewaters are being treated [5]. Biomass loads as high as 150 kg/m^3 are common, compared to 3-4 kg/m^3 in activated sludge and 10-12 kg/m^3 in membrane bioreactors (MBR). Unlike submerged culture technologies, the majority of the biomass in the gills appears to be viable and metabolically active, enabling very rapid nutrient removal.

A second biomass population lives in the decant tank, which is anoxic for most of the batch process, so anaerobes such as denitrifying bacteria and sulphate reducing bacteria are hypothesised to scavenge nitrate and sulphate as alternate electron acceptors for anaerobic respiration. Once the nutrients in the water become depleted the metabolic rate in the gills slows and the gills become an

efficient oxygen transfer device that saturates the water in the decant tank with dissolved oxygen, which prevents denitrification and allows for efficient settling of solids.

Since aeration is via the gills only a small impeller pump is required for recirculation, which makes energy consumption low and maintenance inexpensive and since the technology automatically optimises the biomass composition technical staff are not required to operate the system, making it ideal for developing nations, remote sites and small-scale operations.

The BioGill technology was initially demonstrated at laboratory scale treating settled sewage from a municipal wastewater treatment system using a very primitive form of the BioGill technology [1 and 2]. This is the first report of sewage treatment using BioGill technology at a significant scale (15 m^3/day) using the modern form of the technology.

Methods and Materials

Bio-Gill System Retro-fit

The system was designed to treat up to 15 m^3/day of influent from the kitchen, showers, staff quarters and excess liquid (leachate) from the Clivus-Multrum composting toilets near the main bure, with a BOD of 440 mg/L to produce class-C water for reuse via sub-surface irrigation (Figures 5 and 6).

The existing treatment system (Figure 7A) consisted of a 6.0 m^3 rectangular fibre-glass tank with three main compartments each approximately 2.0 m^3 in volume: 1) primary treatment operating as a conventional septic system with three chambers to promote the separation of solids and an oily scum layer; 2) an aerobic compartment containing coral and a small aeration system consisting of bubblers; and 3) a final settling tank with a small pool filter attached for final treatment. On commissioning the original system failed to even pass a drop of effluent through the filter as the system failed dismally.

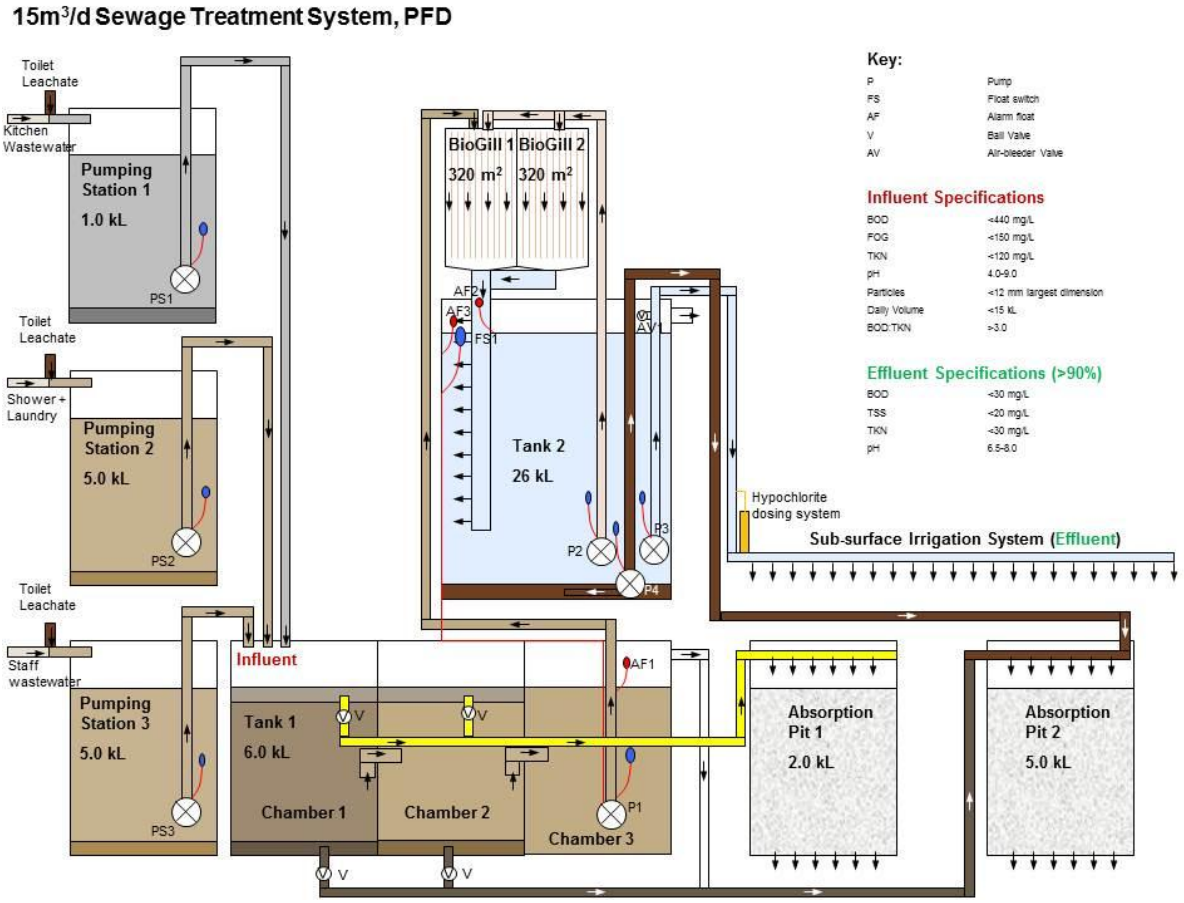

Figure 5: process flow diagram for resort wastewater treatment system.

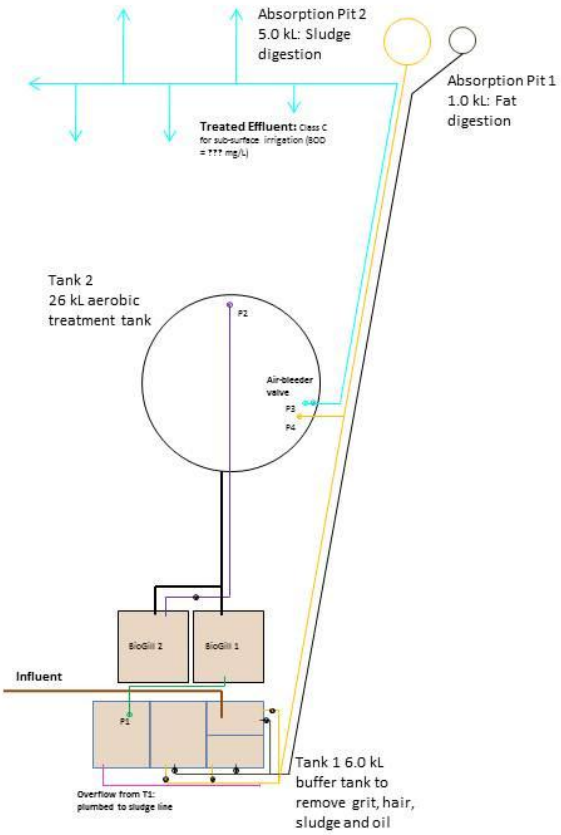

Figure 6: site map for resort wastewater treatment system.

The 6.0 m^3 tank was emptied of the coral medium, the filter and aeration systems removed and the tank was plumbed and retro-fitted according to Figures 5 and 6 so that it would act as a septic system to separate solids and the oily surface scum that separates from the kitchen wastewater (Figure 7A). 50 mm outlets at the top and bottom of each chamber were fitted with manual ball valves to enable the oily surface scum and sludge that settles on the bottom to be drained to absorption pits 1 and 2, respectively. Pump 1 was plumbed and fitted with a float switch to pump 2.5 m^3 aliquots from chambers 3 and 2 via a dispersion manifold into the top of BioGill 1 to transfer the primary-treated water into the aerobic treatment system decanted in Tank 2(Figures 5, 6, 7B and C). Pump 2 recirculated approximately 12 m^3/h through the BioGills for 21 hours and switched off for 90 minutes settling prior to Pump 3 emptying the water in Tank 2 into the irrigation network via a calcium hypochlotite dosing system. Once every week the remaining water in Tank 2 (approximately 3 m^3) was emptied into Absorption Pit 2 via Pump 4. A bypass jet from the hydraulic riser pipe on Pump 4 stirred the sludge during this sludge-removal cycle.

Validation testing was conducted during the peak loading in the winter holidays where the resort had 100 person equivalence (PE) (70 guests and 30 staff living on-site), which coincided with the minimum temperatures for Fiji (20-30^0 C). Therefore, this period represented the most demanding time for the system. The system had been modified by the owners such that it was not performing to specifications and there had been a significant death of the biomass in the BioGills, so the author returned the system to specifications so the validation took place without sufficient ramp-up to have reached peak performance, especially with respect to autotrophic nitrification.

Figure 7: A) pre-existing wastewater treatment system before being refitted to operate as a primary settling tank (Tank 1); **B)** tank 1 after the refit with two 320 m^2 BioGills behind it; **C)** 26 m^3 aerobic treatment tank (Tank 2) located down the hill from the BioGills so that water flowed under gravity from the bottom of the BioGIls back into Tank 2.

To upgrade the system, a hypochlorite dosing system was developed and installed to provide a degree of disinfection for the treated effluent before irrigation. It consisted of a T with a piece of conduit inside it to direct flowing water against a piece of polypropylene shade cloth retaining calcium hypochlorite granules in a 50 mm pressure pipe fitted vertically above it. The pipe had two barrel unions, one below the hypochlorite reservoir to allow access to the conduit and shade-cloth filter and the second at the top to allow addition of the calcium hypochlorite granules.

Testing and Analysis

The system was restored to design specifications and sampling and testing commenced two days later. Samples were collected over the seven-day period following. Samples were tested in the field laboratory for BOD, thermotolerant coliform counts (TCC) and *E. coli* counts, dissolved oxygen, pH, turbidity, free chlorine and temperature. The BOD analysis was performed in new PET soft drink bottles with total volumes of 540 ml, incubated at 27-28^0 C using a Cole-Parmer Instruments Co. 200 mm polarographic dissolved oxygen electrode with a B&C Electronics OD 7685 microprocessor. The increased incubation temperature was expected to increase the amount of oxygen consumed by a given sample relative to the procedure performed at 20^0 C and using the rule of thumb that biochemical reaction rates in the mesophilic range double with an increase of 10^0 C, the catabolism would have consumed nearly all of the biomass accumulated in a normal 5-day incubation, so the result would approach 35% higher than the same test performed at 20^0 C. The TCC and *E. coli* counts were incubated at 41.5-42.6^0 C next to the diesel generator, which was acceptable for the test to be sufficiently accurate. Where the water was heavily contaminated 0.1 ml aliquots were plated and spread with a sterile spreader. Where the water was considered clean, 100 ml samples were filtered onto glass-fibre filter discs and plated as normal. Gridded cellulose nitrate disc filters could not be transported on the plane as they are explosive. Samples from various parts of the treatment system were immediately tested for dissolved oxygen using the same oxygen electrode. The pH was analysed using a Cole-Parmer Instruments Co. NK435 250 mm refillable pH probe with a Jenco 86309 PDT microprocessor and the turbidity of the samples were tested using a Hanna Instruments HI93703 turbidity meter. Where the samples were chlorinated, the free chlorine concentrations were determined using a Eutech Instruments ECC201 free chlorine meter. Samples were also chilled and sent to National Water Quality Laboratory (NWQL) in Suva to be tested for BOD (2510B), total suspended solids (TSS) (2540C), faecal coliform counts (9222D), total organic nitrogen (TON) (4500-NorgA), ammonical nitrogen (4500-NH_3G), nitrate (4500-NO_3^-) and total reactive phosphates (TP) (4500PE) using standard procedures to augment the array of testing performed and to act as a reference to compare with the data acquired in the field under less than ideal conditions. The act of chilling and storing samples was expected to have altered the data in the following ways: 1) the oily components in the Tank 1 (T1) influent samples would have stuck to the hydrophobic sample bottles, especially at lower temperatures, so the BOD of these samples as determined by the NWQL would have been significantly reduced; 2) clumps of actively growing coliforms would have dispersed as a result of metabolic dormancy so the faecal coliform counts would have been elevated; and 3) nitrates would have been consumed by denitrifying bacteria under the anaerobic conditions during storage, albeit slowly, so older samples were expected to have lower nitrate levels than fresh ones.

There was a misunderstanding in the allocation of tests to be performed on the samples with respect to nitrogen analysis, so TON was tested for instead of TKN. To correct this, three composite samples were taken a month later from the Tank 1 influent and Tank1 effluent sampling sites and tested for TON (4500-NorgA), ammonical nitrogen (4500-NH_3G), nitrite (4500-NO_2^-), nitrate (4500-NO_3^-) and total reactive phosphates (TP) (4500PE). The average TON and ammonical nitrogen ratios were calculated for the various samples and applied to the TON data to estimate the ammonical nitrogen and TKN for the data collected in this trial.

Hydraulic retention time (HRT) was calculated as follows: 1) primary treatment was calculated by dividing the volume of Tank 1 (6.0 m^3) by the total volume treated in the batch multiplied by 24 hours; and 2) since the arrival of influent was random between the hours of 06:00 and 20:00 treatment time in Tank 2 was calculated as half of the present time minus 6.00 hours, and from 20:00 onward to 06:00 the next day each hour was counted as one hour of treatment time. This gave the secondary treatment an average batch HRT of 17.0 hours. The HRT for primary treatment was added to that for secondary treatment to give the HRT recorded in graphs and tables.

Results and Discussion

Even though the biomass on the gills had only two days to ramp-up before testing commenced, the water quality coming out immediately after restoration to specifications was remarkably good, (Table 1 and Figures 8 and 9). These results are consistent with the observation of rapid BOD removal from various wastewaters by BioGill wastewater treatment systems, especially those containing high levels of fats, oil and grease [4-6]**.** The average BOD of the influent fell from 662mg/L to 320 mg/L with an average HRT in the primary treatment system of 14.4 hours, which was very rapid for an anaerobic settling system (Table 1 and Figures 8 and 9), considering that the grease-trap on the kitchen was operating to specifications and very little settled solids were collected from the primary treatment tank. The pH consistently neutralized in the primary treatment system. There was a noticeable accumulation of a greasy scum layer on the top of the primary tank, which appears to have been due to a reaction between free fatty acids and ammonium ions yielding fatty acid amides and/or ammonium soaps. Roe *et al.* [8] determined that fatty acid amide yields at 25^0 C were undetectable, but extensive ammonium soap formation was detected at ambient temperatures. Therefore, the extensive scum layer formation and BOD removal from the water in the primary treatment system with the concurrent loss of one third of the turbidity, the neutralization of the pH and reduction in the characteristic rancid odour of biodegrading free fatty acids was most likely due to ammonium soap formation (Table 1 and Figures 8 and 9). Lipase activity was also considered as the cause, but lipases are produced aerobically and the resultant acetic and propionic acids would have lowered the pH, not raised it, so it was concluded that lipase activity could not account for the changes detected.

Table 1: Mean and (standard deviation) for various physical, chemical and biological parameters of the wastewater at various treatment stages in a BioGill reactor treating resort wastewater. Abbreviations used include: hydraulic retention time (HRT), biochemical oxygen demand (BOD), total suspended solids (TSS), free chlorine (Free-Cl) total organic nitrogen (TON) total Kheldahl nitrogen (TKN), ammonical nitrogen (NH_4^+), nitrate (NO_3^-) and total reactive phosphate (TP).

Parameter	Units	Tank 1 Influent	Tank1 Effluent	Tank 2 Effluent
Volume	m^3	13.3 (3.4)	13.3 (3.4)	13.3 (3.4)
HRT	hours	0 (0)	10.8 (4.2)	27.8 (4.2)
BOD	mg/L	662 (151)	320 (50)	24.6 (6.3)
BOD removed	%	0	48	96
TSS	mg/L			9.6 (5.1)
Turbidity	NTU	300.0 (93.4)	202.3 (47.8)	18.7 (2.2)
pH		4.94 (0.74)	6.97 (0.81)	7.29 (0.20)
Free-Cl	mg/L			0.32 (0.25)
Faecal Coliforms	CFU/100 ml			412 (345)
E. coli	CFU/100 ml			81
TON	mg/L	72.90 (31.90)	62.45 (17.35)	20.07 (1.92)
NH_{4+}	mg/L	39.92*	44.13*	1.92 (0.57)
TKN	mg/L	112.1*	105.8*	21.99
NO_3^-	mg/L			2.52 (4.63)
TKN removed	%	0	5.8	80.4
BOD:TKN		5.9	3.0	1.1
TP	mg/L	3.88	4.33	4.01

BOD removal in the aerobic treatment stage was also very rapid (Table 1 and Figure 9A), with the BOD falling on average from 320 mg/L to 24.6 mg/L, representing a 1.11 log reduction with an average aerobic treatment HRT of 17 hours. The three pumping stations around the site did not have timers to coordinate them with the batch process of the treatment system, causing the nutrient levels to rise in Tank 2 late in the batch (Figure 9). All of the batches tested fell to approximately the same BOD indicating that they too were subject to late influx of influent from the kitchen because the pumping station was only 1.0 m^3 in volume and the aliquots that it pumped were only about 250 L each. It has also been observed in previous studies of the BioGill technology [5 and 6] that after a single log reduction BOD removal rates slow as the biomass shifts from growth (catabolism and anabolism) to maintenance energy (catabolism only) and some microbes commence secondary metabolism, resulting in autolysis. From these observations the strategy was formed to use multiple stages to separate copiotrophs from oligotrophs into two separate systems. To achieve lower BOD concentrations from such rich influent to make class A effluent with BOD and TSS <10 mg/L a second aerobic treatment stage would be necessary [5 and 6].

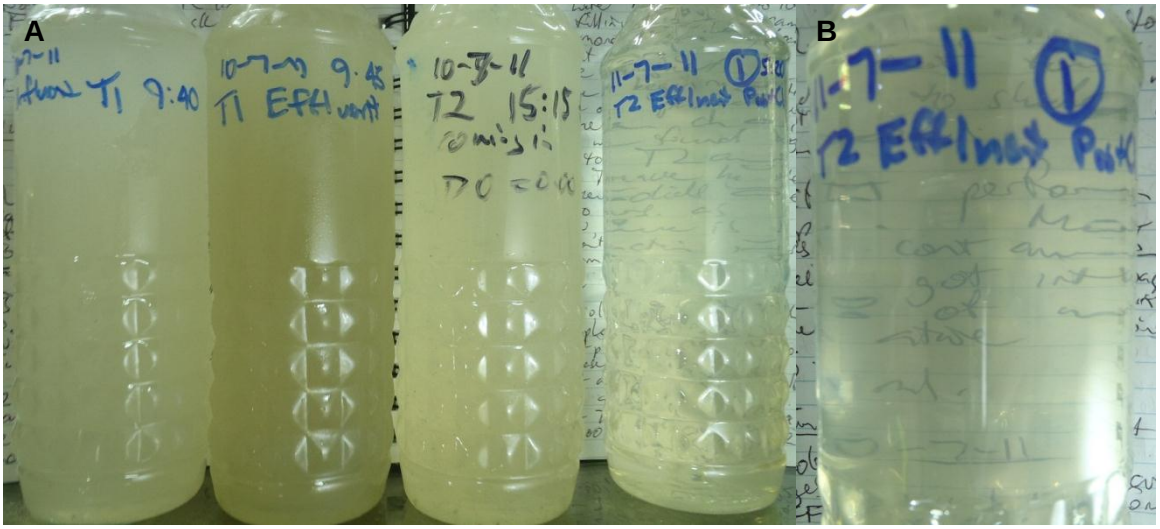

Figure 8: A) Water sample taken from the treatment system. Left to right are Tank 1 influent, Tank 1 effluent, Tank 2 during treatment and Tank 2 final effluent; and **B)** close up of the final effluent from Tank 2.

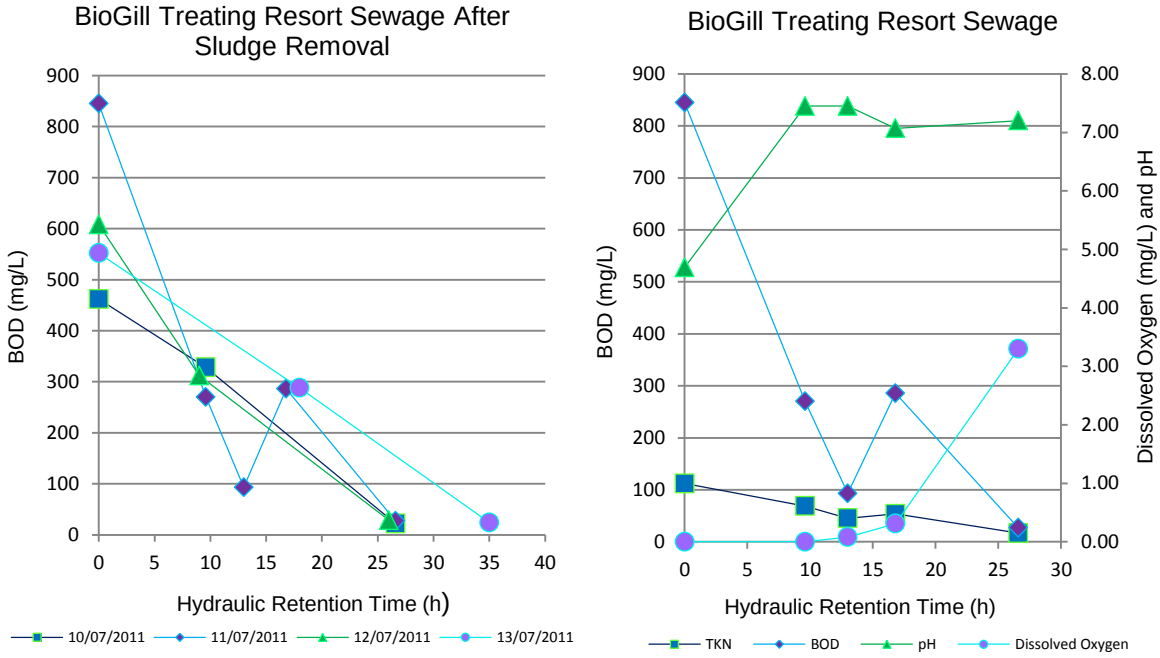

Figure 9: A) Plots of biochemical oxygen demand (BOD) versus hydraulic retention time for the BioGill system treating resort wastewater. Note the spike in the BOD late in the batch due to the kitchen wastewater pumping station not being coordinated to operate with the treatment system, after which the BOD was rapidly reduced from 286 mg/L to 28.5 mg/L in 9.8 hours. **B)** Plot of total Kjeldahl nitrogen (TKN), biochemical oxygen demand (BOD), pH and dissolved oxygen concentration versus hydraulic retention time for the BioGill system treating resort wastewater for a single batch. Note the rapid neutralization of the acidic influent with concurrent reduction in BOD and TKN under anaerobic conditions. Also note the late rise in dissolved oxygen concentration signalling the shift from a denitrifying decant tank to an aerobic system that aided the settling of solids.

Nitrogen removal was monitored at various sites (influents, treatment stages, effluent and groundwater entering the reef) to determine the impact of the treatment system on the availability of nitrogen in its various forms on the gardens and reef around the resort. The Clivus-Multrum composting toilets (Figure 10A) were found to be a very significant source of pollution in the environment, as they leach significant quantities of an extremely concentrated nitrogenous wastewater composed of a slurry of urine and faeces that drains into the sand (Figure 10C). The drain is described in the technical specifications as an “overflow” [9] but it is at the bottom of the tank, so we refer to it as the “leachate drain”. The composting toilets are claimed to dehydrate all of the

liquids via the flow of air through the compost/solids, but from examination of the leachate entering the pumping station (Figure 10B), it is evident that significant quantities are discharged without treatment from the system directly into the adjacent soil, which eventually renders the soil toxic and creates a strong odour of ammonia and butyric acid. From the data in Table 1 it was estimated that an average of 60-80 L/day during peak occupancy was being drained from these toilets into the wastewater treatment system, but a further six small-scale composting toilets with a person equivalent (PE) of 12, were not connected to the sewerage system, so their leachate albeit significantly less in volume still flows into the sand, which drains into the groundwater. The leachate had an average BOD of >5600 mg/L and the TON was > 16700 mg/L which is consistent urine having 1-2% nitrogen [10]. The TON:TKN ratios determined in the Tank 1 influent samples collected a month later were 0.65, indicating that the TKN for the leachate could have been as high as 25,700 mg/L, which is consistent with partial drying of the urine.

Figure 10: A) Clivus-Multrum composting toilet under a bure; **B)** the outlet where the leachate enters the pumping station beside the main toilet block. Note the wide stain from the peak flows during the night when the Beach Bar is heavily occupied; and **C)** a sample of the leachate.

The estimated volume of leachate calculated from the nitrogen concentrations in the wastewater were consistent with the estimate of 70 guests producing about 2.0 L/day each, given that most were drinking alcohol, coupled with partial air drying in the composting toilet. The BOD:TON ratio was 0.34 and the estimated BOD:TKN ratio was 0.22. Various authors report that BOD:TKN ratios of sewage become carbon-limited below 2.5-3.0 [11 and 12] and incomplete denitrification occurs, so the notion of treating the leachate alone poses significant problems with respect to nitrogen removal.

The influent entering the primary treatment system had TON of 72.9 mg/L and an estimated TKN of 112.1 mg/L, with a BOD:TKN ratio of approximately 5.92, and after leaving the primary treatment system the TON was 62.5 mg/L and the estimated TKN was 105.8 mg/L (Table 1). The BOD of raw sewage is typically 200 mg/L and the TKN generally equals 30-40 mg/L [12], so the BOD:TKN is usually about 5.0-6.7. The BOD in the influent at the resort was on average 3.3 times stronger and the TKN was 2.8 times stronger than raw sewage, which is consistent with the water conservation measures used on the island.

Approximately 6.3 mg/L of nitrogen appears to have been removed in primary treatment, which is consistent with the theory that the scum on the surface of Tank 1 was formed from the precipitation of ammonical soaps from the reaction of ammonium ions with free fatty acids [8]. The free fatty acids mostly came from the kitchen wastewater and the ammonium ions came from the biodegradation of urea from the main toilet block. Using oleic acid as a representative of free fatty acids with a molecular weight of 282 g/mol and assuming that ammonium ions react with oleic acid in a stoichiometric ratio of 1:1 to from ammonium oleiate soap, the loss of 6.3 mg/L of nitrogen as TKN would be sufficient to remove 127 mg/L of oleic acid, which has the BOD conversion factor of 2.95 and is equivalent to 374 mg/L of BOD, which is consistent with the data recorded in Table1 where about 340 mg/L of BOD was removed in approximately 11 hours under anoxic conditions without a significant alternate electron acceptor. This appears to be a very simple, but efficient mechanism for removing free fatty acids from wastewaters such as kitchen wastewater. Waterless urinals could be connected in restaurants to the kitchen wastewater line prior to the grease-trap to optimise recovery

of fats. Creating an aerobic retention zone to promote the growth of urease positive microbes and subsequent ammonification would further increase the efficiency of the removal of free fatty acids as ammonical soaps in the grease-trap downstream.

Ammonia oxidation and denitrification caused a slight lowering of the pH as ammonia was oxidised and removed as nitrogen gas (Figure 9B), indicating that the denitrification process was returning alkalinity, however, the TKN removal rate slowed toward the end of the batch, indicating that ammonia oxidation was becoming retarded, despite almost complete denitrification taking place (Table 1). The TON, ammonical nitrogen, and nitrate levels in the effluent demonstrated that the vast majority of nitrogen was locked up as organic nitrogen, indicating that biodegradation of the organic nitrogen was the rate limiting factor or autolysis from the biomass in the system was releasing significant amounts of nitrogen back into the system. The dissolved oxygen rose at the end of the batch, which facilitated efficient settling of solids, with TSS averaging 9.6 mg/L (Table 1). This demonstrates that the system is very well balanced with respect to the form factor (membrane area divided by batch volume) and the loads placed on it. More membrane area would cause more extensive BOD removal and organic nitrogen biodegradation, but the dissolved oxygen levels would rise earlier in the cycle possibly causing incomplete denitrification as was observed in earlier trials [2].

Literature about wastewater treatment provided a great degree of disparity between data reported in journals and reports in online websites. The Wright's Training Site reports that in general, secondary treatment systems remove up to 30% of total nitrogen [12]. Lesjean *et al.* (2002) [13] reported a comparison between a well-operated conventional wastewater treatment plant (WWTP) with two small-scale MBR pilot-plants. Performance data from the best performing MBR configuration is listed in Table 2 along with the Ruhleben WWTP, which consistently removes 84% of the total nitrogen (TN) from municipal wastewater [13]. It appears that the best results are published in peer-review journals, while poorer results do not make the grade, which means that looking to peer-review journals for indicative or typical data for comparison can be misleading. In a survey of 29 WWTP in the United Kingdom, seven failed to produce effluent with COD below the 125 mg/l limit and 20 failed the standard for 75% COD-removal [14]. In the report, most WWTP did not have sufficient data in their records for their performance to be analysed, so the actual failure rate is expected to be much higher. Municipal wastewater usually contains a higher COD:BOD ratio (2.0-4.5) than the conversion factor of 1.5 used to estimate the COD of the resort wastewater treated in this study, as reported in Table 2. This is because all of the cellulose from toilet paper was removed in the composting toilets, so the lower figure representing 100% biodegradable compounds was selected to estimate the COD. Settling of cellulose (toilet paper) is simple and quick in primary treatment, which elevates the performance of the Ruhleben WWTP in terms of COD removal compared to the BioGill treatment system reported here. This makes it difficult to accurately compare the BioGill system to other systems in Table 6 due to the different testing procedures used and the different influent. The BOD removal rate in the BioGill system was approximately twice that recorded in an advanced secondary treatment system in an Australian municipal WWTP [2] and the nitrogen removal rates were very rapid, even compared to the Ruhleben WWTP (Table 2). Mota *et al.* (2005) reported effluent from intermittently aerated reactors with HRT of 3 days using anaerobically digested swine wastewater with COD 344 mg/L, TKN 296 mg/L and NH_4^+ 197 mg/L as the influent and the effluent had NH_4^+ concentrations of 22-32 mg/L [18]. The nitrogen removal rate was 88-91 g/m^3.day in their heavily-loaded system, which was slower than 115 g/m^3.day recorded in the BioGill system operating with much lower loading rates.

Solids-settling was also very efficient in the BioGill system, due to the system's natural disposition to balancing the removal of BOD with ammonia oxidation and denitrification and the concurrent aeration with settling. It appears likely that elevated catabolism in the BioGills [3 and 7] produced relatively high levels of CO_3^{2-} in the biofilms on the gills, which in turn promoted autotrophic nitrification (Figure 11), this was coupled to almost complete denitrification that took place concurrently in the anoxic decant tank below and led to very efficient nitrogen removal and settling of solids. The BioGIll system demonstrated that all of these functions are naturally optimised within it, which engineers struggle to achieve using submerged-culture technologies as described by Jeyanayagam (2005) [11]. It should also be emphasised that the system was recovering from the biomass being killed by starvation, so nitrification and BOD removal were expected to become even more efficient after a ramp-up period and the performance clearly improved through the course of this short trial.

Table 2: reported performance data for various wastewater treatment processes at different scales compared to data collected in this study.

Parameter	Units	Study							
		Lesjean *et al.* (2002) [13]	**Lesjean *et al.* (2002) [13]**	**Ahn *et al.* (2003) [15]**	**Pynaert *et al.* (2003) [16]**	**Hiras *et al.* (2004) [17]**	**This Paper**		
Bioreactor type(s)		Conventional WWTP (240,000 m^3/day)	Pilot-scale anaerobic /aerobic /anoxic MBR (3 m^3/day)	Sequencing anoxic/ anaerobic MBR (SAM) and modified Luzack-Ettinger MBR (MLE)	Lab-scale autotrophic rotating biological contactor (RBC)	Lab-scale two-stage RBC	Primary settling tank	BioGill reactor	Whole system (15 m^3/day)
Wastewater		Municipal wastewater	Municipal wastewater	Sewage spiked with glucose and acetic acid	C-free NH_4^+ salts simulant	Municipal sewage	Resort sewage	Settled resort sewage	Resort sewage
HRT	hours	18	18	24	17	24	10.8	17	27.8
BOD influent	g/m^3				0	382	662	320	662
COD influent	g/m^3	998	740	245	0	618	993*	480*	993*
TN influent	g/m^3	69.7	61	37.5	840	61	NR	NR	NR
TKN influent	g/m^3	NR	NR	NR	840	61	112	106	112
BOD effluent	g/m^3	NR	NR	NR	0	53	320	25	25
COD effluent	g/m^3	56	35	10 (SAM) 4 (MLE)	0	112	480*	37.5*	37.5*
TN effluent	g/m^3	11.5	3.6	15 (SAM) 12.2 (MLE)	93	28		24.5	24.5
TKN effluent	g/m^3	4.6	2.6	NR	38	9.1	106	21	21
N oxidised effluent	g/m^3	6.9	1.0	NR	55	19		2.5	2.5
Hydraulic loading	L/ m^2.day	NR	NR	NR	9.9	12	NR	21	21
Biomass loading	kgMLSS/m^3 kg/ m^3	3-5	10-12	10-11	NR	NR	NR	18-22**	18-22**
BOD loading	g/m^3.day	NR	NR	NR			1471	471	572
	g/m^2.day***				0	5.9		6.7	6.7
COD loading	g/m^3.day	1331	NR	245			2207	677	858
	g/m^2.day***				0	9.2		10.0*	10.0*
TN loading	g/m^3.day	92.9	NR	37.5	1189	0.41	249	150	97
	g/m^2.day***				8.3	0.99		2.3	2.3
BOD removal rate	g/m^3.day	NR	NR	NR	0	329	758	416	550
	g/m^2.day***				0	4.0		8.7	
COD removal rate	g/m^3.day	1256	NR	NR	0	506	1137*	624*	825
	g/m^2.day***				0	6.1		13.1*	
TN removal rate	g/m^3.day	58.2	57.4	22.5	1158	33	13.3	115	75.5
	g/m^2.day***				8.3	0.4		1.7	
BOD removal efficiency	%	NR	NR	NR	0	86	52	92	96
COD removal efficiency	%	94	96	96 (SAM) 98 (MLE)	0	82	52*	92*	96*
TN removal efficiency	%	84	94	60 (SAM) 67 (MLE)	89	54	5	77	78

NR not reported

* COD estimated using COD:BOD ratio of 3:2

** biomass loading in BioGill reactor calculated from an estimated biofilm thickness of 1.5-1.8 mm x 640 m^2 to give the wet mass and divided by 4 to give an estimate of the dry mass.

*** loading rates for rotating biological contactors are recorded in terms of biofilm area g/m^2.day and loading rates for BioGills are recorded in terms of hydraulic volume and membrane area g/m^3.day and g/m^2.day

Biological nitrogen removal via oxidation of organic nitrogen and ammonium ions has been well studied and various biological mechanisms have been reported, including: 1) autotrophic bacterial nitrification [15, 19-21]; 2) heterotrophic bacterial nitrification [22 and 23]; 3) heterotrophic fungal nitrification [24-31]; 4) anaerobic bacterial denitrification [15 and 19]; 5) aerobic bacterial denitrification [22 and 23]; and 6) anaerobic ammonia oxidation [19].

Autotrophic ammonia oxidising bacteria are very fastidious obligate aerobes that do not nitrify under acidic conditions (<6.5) in wastewater treatment systems. As autotrophs they rely in single-carbon molecules (e.g. carbonate, methanol and methane) to grow, making their cultivation in the highly variable mixed-culture environment of a wastewater treatment system, very difficult. It has been demonstrated that they are protected from low pH (e.g. 3.2) in high population densities associated with particulate matter where they continue to nitrify. *Nitrosomonas europea* grown as biofilms on glass beads had no lag period for ammonia oxidation, whereas the same culture grown in liquid medium had a significant lag period [21]. This phenomenon may partially explain the immediate

nitrification detected in the BioGills after nutrient supply to the majority of the gills was blocked for weeks and then resumed.

Pynaert *et al.* (2003) reported a purely autotrophic rotating biological contactor (RBC) operating without any carbon load and a very high NH_4^+ load that was ramped up for 550 days before the experimental data was collected [16]. Their system removed nitrogen at 3.0 times the rate of the BioGill system (m^2:m^2) described in this study at 7.9 times the nitrogen load (Table 2), so it is very difficult to compare the two systems, as the autotrophic nitrogen-removing biofilm had no competition from heterotrophs and the influent was consistent in composition and quantity. This report demonstrates the theoretical limits for nitrogen removal using RBC technology.

Gieseke *et al.* (2006) reported nitrification at low pH in biofilms growing on chalk particles as determined using microelectrodes. The authors hypothesised that the cation exchange capacity confired a buffering effect on the cells in the biofilms [20]. Tarre and Green (2004) demonstrated that autotrophic nitrification can occur at low pH in biofilms growing on sintered glass particles [21]. They also demonstrated that there is no lag for the onset of nitrification under such conditions, whereas the same culture grown in liquid culture had a significant lag before nitrification commenced. The cation exchange capacity of the particle surfaces (glass, clay, chalk etc.) appears to have provided a pH buffer equal to 2 pH units as NH_4^+ absorption acted as the buffer [20 and 21]. The rapid revival of nitrification in the BioGills without a significant ramp-up period, seen in this study, was inconsistent with the very slow growth of autotrophic nitrifying bacteria. This result could be explained by the silica in the BioGill membranes providing autotrophic nitrifying bacteria with the ability to survive without nutrients for extended periods of time.

Heterotrophic nitrification is important in acidic conditions (coniferous forests etc.), where there is a large heterotrophic biomass and where the C:N ratios are high [23]. Heterotrophic nitrification appears to fix a the metabolic bottleneck in electron transport that occurs during mixotrophy by reducing NAD(P)H back to $NAD(P)^+$[22]. Various species perform this function when there is a lack of reduced sulphur, particularly thiosulphate. Although the specific heterotrophic nitrification rates by individual cells are significantly lower in heterotrophic nitrifiers compared to autotrophic nitrifiers, large heterotrophic populations can account for the majority of nitrification in many environments. In early works it was demonstrated that reduced sulphur compounds are rapidly oxidised in the BioGills [2], as sulphate concentration reaches a plateau within 3.0 hours of operation, in this respect conditions in the BioGills as ideal for heterotrophic nitrification to take place, which could account for the lack of a lag period and rapid rate of nitrogen removal from the BioGill system without significant accumulation of nitrate.

Aerobic denitrification appears to be linked to heterotrophic nitrification and is the result of mixotrophy with acetate and nitrate when acetate is limited. *Thiosphaera pantotropha, Alcaligenes* sp., *Pseudomonas denitrificans, A. faecalis* and *P. aeruginosa* conduct aerobic denitrification when the dissolved oxygen concentrations are >50% of saturation and *Zoogloea ramigera*, *P. stutzeri*, *Hyphomicrobium* sp. and *P. fluorescens* denitrify with the dissolved oxygen concentration between 15-35% [22].

Doxtader and Alexander (1966) demonstrated that *Aspergillus flavus* grown as a surface culture oxidised ammonium ions to nitrate via 3-nitropropanoic acid [24]. Old cultures (>14 days) did not nitrify reduced nitrogenous compounds. Schimel *et al.* (1984) used acetylene to block autotrophic NH_4^+ oxidation and chlorate to block autotrophic nitrification to elucidate heterotrophic nitrification in the soil [25]. They also used $^{15}NH_4^+$ to demonstrate that the heterotrophic did not occur via the oxidation of NH_4^+. The fungicide cycloheximide partially inhibited nitrate production and streptomycin did not inhibit nitrate production at all, so most of the nitrification was due to fungal nitrification. *Aspergillus flavus* was not inhibited by acetylene and it continued to nitrify organic nitrogen to nitrate without nitrite as an intermediate. Killham (1990) detected nitrification in acidic soils as being predominantly fungal in nature [26]. Kuyper and Bokeloh [27] demonstrated that the Basidiomycete *Clitocybe metachroa* conducts ligninolysis and nitrification concurrently. They demonstrated that NH_4^+ and simple organic nitrogen compounds are oxidised to nitrate possibly by superoxide radicals from peroxidase enzymes. This process does not yield energy for the organism and is widespread among ascomycetes and deuteromycets. Fungal nitrification is hypothesised to be mediated via hydroxyl free radicals in the presence of hydrogen peroxide and superoxide produced by fungal peroxidase and oxidase enzymes [19]. Guest and Smith (2002) [15] reported that some fungi can nitrify faster

than bacteria and are less fastidious than their bacterial counterparts. The prevalence of fungi in BioGills indicates that fungal nitrification may have also occurred in this trial, which can also explain the rapid onset of nitrification after starvation. It appears most likely that autotrophic nitrification, heterotrophic nitrification and fungal nitrification took place in the gills and denitrification took place in the decant tank below (Figure 11).

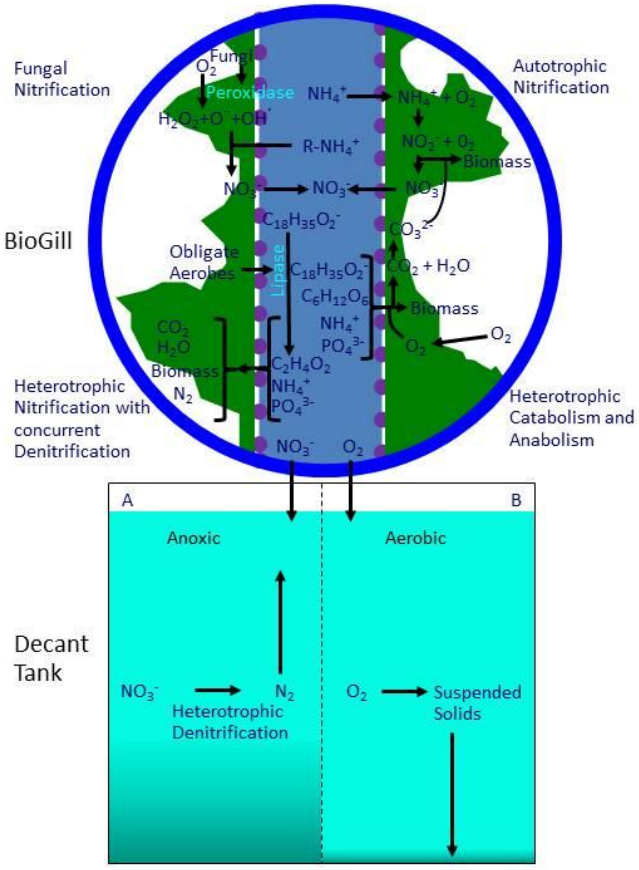

Figure 11: Schematic diagram of the various forms of nitrogen metabolism hypothesised to take place in the BioGill treatment system. Note the relationship between heterotrophic catabolism and autotrophic nitrification to balance the required alkalinity in the form of carbonate ions and the sequential processes in the decant tank that first remove nitrogen via anoxic denitrification and then remove solids via aerobic settling. Enzymes are labelled in aqua.

To estimate the total amount of nitrogen removed by the system per year, the daily nitrogen removal during peak occupancy was calculated from the data in Table 1 to average 1.224 kg/day. Peak occupancy (70 guests and 30 staff) occurs for about three months per year and the remaining nine months has about half occupancy (20 guests and 30 staff), so the wastewater treatment system removes about 280 kg of nitrogen per year, which is equivalent in nitrogen content to 5.6 tons of blood and bone fertiliser, so the treatment system definitely has a significant impact on the total nitrogen entering the reef.

TP concentrations in the influent and the effluent were about the same (Table 1), so no net phosphorous removal was detected. The phosphorous levels in the influent were low and would have made other biological treatment systems struggle, as when the C:N:P ratio is below 100:10:5 other technologies become rate limited, as they rely of the conversion of nutrients to solids via anabolism to remove 80% of the nutrients, whereas it appears that the BioGill technology relies more heavily on catabolism, as it produces significantly less biomass and much greater quantities of CO_2, which would dissolve as carbonate (CO_3^{2-}) [7]. Since fungi predominate the biomass, they do not require as much N and P to grow, as their cellwall is made of cellulose (COH) not amino sugars and polypeptides (peptidoglycan), they have much less plasma membrane area per unit volume or gram of dry weight, as the cells have drastically smaller surface area:volume ratios, and because of their size they also have about 2% as much DNA/g of dry weight, which reduces the amount of phosphorous and

nitrogen needed to grow. Assuming that the BOD:carbon ratio was 2.0 for the wastewater (a balance of fats and carbohydrates) the average C:N:P ratios for the Tank 1 influent, Tank 1 effluent and Tank 2 effluent were 100:33.9:1.2, 100:66.5:2.5 and 100:163.2:16.3, respectively, so the water entering the aerobic treatment system would have significantly retarded treatment using submerged bacterial cultures that predominate the biomass in all other aerobic treatment technologies. This demonstrates the significance of having fungal biomass over bacterial populations.

The annual rain fall in Fiji is focused into the wet season, so for half of the year the rainfall is insignificant, which makes growing and maintaining gardens difficult without a supplementary water supply. When the wastewater treatment system was commissioned the gardens at the resort were relatively sparse as can be seen in Figure 12A. Within less than nine months of irrigating with the effluent from the treatment system the gardens sprang up all over the resort as can be seen in a photo of plantation hill in Figure 12B. There was a good wet season on the island in that period, but the irrigation water with low doses of nitrogen as nitrate and organic nitrogen would have played a significant role in promoting the extremely rapid growth known among staff as the "instant jungle". The unirrigated areas in the background of the two photos showed little change, but the same plant species grew rapidly down between the Bures where they were irrigated.

Figure 12: A) The BioGill wastewater treatment system at the time of commissioning; and **B)** the same hill eight months after. The staff at the resort called it the "instant jungle".

Samples of the groundwater exiting the resort from the sand at the low-water mark (Figure 13) showed low TON (0.57 mg/L) and nitrate levels (1.6 mg/L), well within the Fijian standard for environmentally sensitive areas (TON < 10 mg/L). The TKN for marine water is usually about 0.5 mg/L [32], so the nitrogen in the groundwater was not significantly elevated over this level. The algal margin had receeded from being 10-15 m wide before the commissioning of the treatment plant to being only 5 m wide in front of the beach bar, where the vast majority of the nitrients are released via the irrigation system, compost on the gardens and the two absorption pits. The region where the algal bloom has receded from has small coral outcrops growing from brachiated corals washed in from the adjacent established parts of the reef and is predominated by staghorn corals and some small brain corals. The author considers that the large amounts of nitrogen that the treatment system removes from the resorts wastewater has had a significant role in this improvement. The elevated rainfall would have also contributed as it would have diluted the nutrients in the groundwater, reducing the availability of nitrate for algal growth.

The reef in front of the resort is stunning to say the least and the proximity to accommodation and facilities provides a unique opportunity seldom found elsewhere in the world. This makes this unique site of ecological significance and of great value to the resort. In so protecting it is of the utmost importance for the sustainability of this ecotourism operation. The biodiversity seen within 100 m of the beach was exceptional as can be seen in Figures 14 and 15. The species identifications were performed using several publications as guides [33-35] and this small list should not be considered in to be exhaustive. The author estimates seeing >100 vertebrate species and >150 invertebrate species in 10 hours of snorkelling in 0.5 hectare of reef.

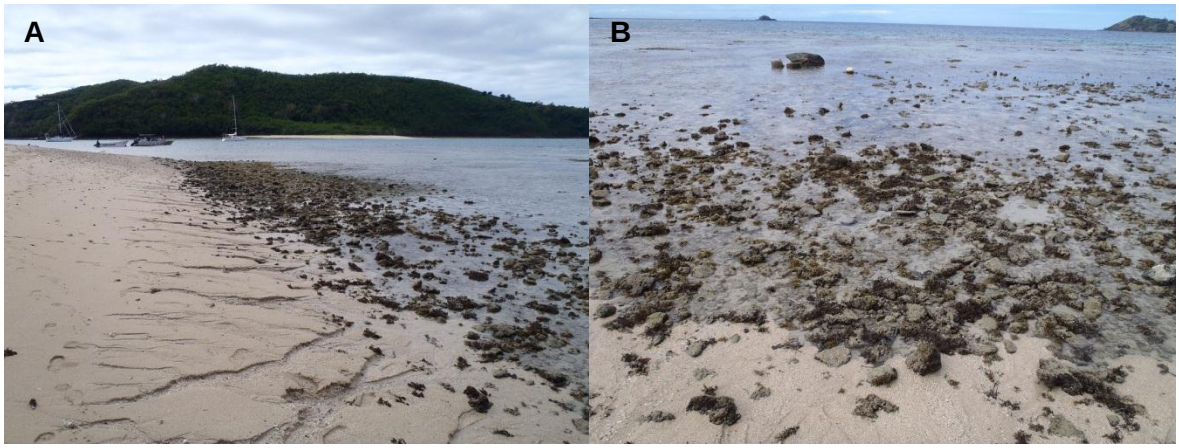

Figure 13: A) Low tide in front of the Beach Bar showing the algal bloom (centre) and groundwater rivulets in the sand; and **B)** the receding algal bloom at the low-tide line with small brecciated coral starting to repopulate about five meters out.

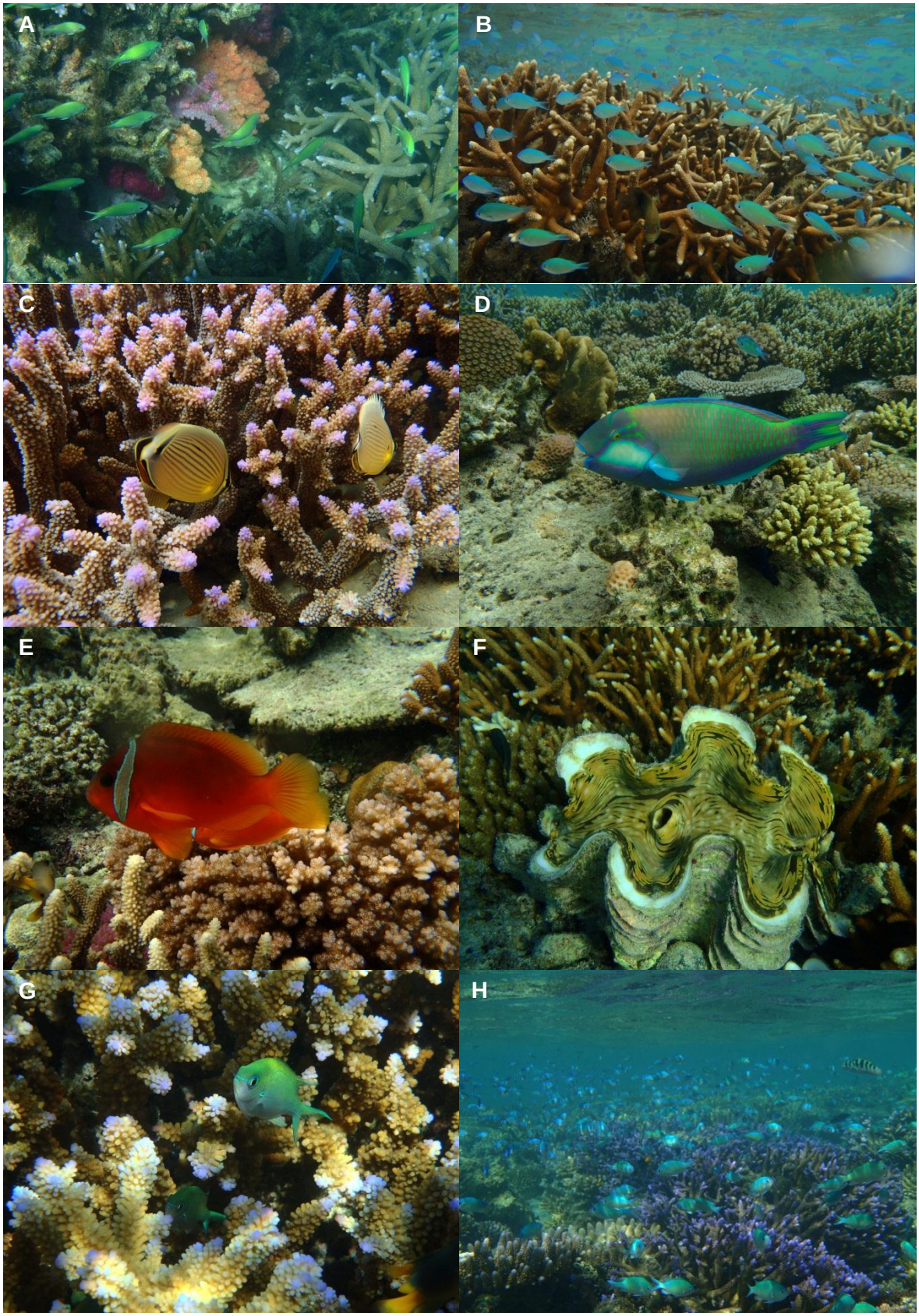

Figure 14: A) Green Pullers (*Chromus viridus*) **B)** blue-green pullers (*Chromus atripectoralis*); **C)** oval butterfly fish (*Chaetodon* lunulatus); **D)** parrot fish; **E)** spine-cheeked anemone fish (*Premnas biaculeatus*); **F)** giant clams; **G)** a multitude of corals; and **H)** shoals of reef fish were seen less than 100 m from the beach directly in front of the resort.

Figure 15: A) Oblique-banded sweetlips (*Plectorhinchus lineatus*) and horned bannerfish (*Heniochus varius*); **B)** lagoon trigger fish (*Rhinecanthus aculeatus*); **C)** ridge coral; **D)** triangular butterfly fish (*Chaetodon baronessa*); **E)** feather star (*Himerometra* sp.); **F)** orange-fin anemone fish (*Amphiprion chrysopterus*); **G)** shoals of convict tang (*Acanthurus triostegus*); and **H)** saddled butterfly fish (*Chaetodon auriga*) were among the plethora of biodiversity.

Conclusions

The wastewater treatment system was designed to treat much lower nutrient concentrations in the influent (BOD 440 mg/L not 662 mg/L) and smaller hydraulic volumes (15 m^3 not 16 m^3) (Figure 5). Once the system was restored to factory specifications it performed exceptionally well with respect to removal of BOD, nitrogen and suspended solids. No net phosphorous removal was detected, but the phosphorous levels were extremely low in the influent, so no removal was needed to meet Fijian standards. After nearly nine months of operation there was no sign of membrane fouling or deterioration and no decrease in treatment performance consistent with all other BioGill systems operated so far [1-6]. This demonstrates that the technology is simple, reliable and economical as the energy consumption is approximately 1.45 kWh/m^3 treated. The simplicity of operation and maintenance makes this technology suitable for remote sites and developing nations and it out-performs most large-scale WWTP without the need for technical staff to operate it.

Future research into the nature of the nitrogen metabolism processes using fluorescent *in situ* hybridisation, metabolic inhibitors such as acetylene and chlorate and ^{15}N labelled substrates to determine the nature, location and magnitude of the various contributors to the removal of nitrogen would facilitate understanding and optimisation of biological nitrogen removal using the BioGill technology.

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