FIRST INTERNATIONAL SEMINAR
On Algal Technologies for Wastewater Treatment and Resource Recovery
Pictures on the cover courtesy Andres Alfonso Martinez
WELCOME TO UNESCO-IHE

Dear participant to the first International Seminar on Algal Technologies for Wastewater Treatment and Resource Recovery. I would like to welcome you to UNESCO-IHE Institute for Water Education, and wish you an inspiring visit! The programme of the seminar includes presentations on innovative ways to treat wastewater in an environmental friendly manner. Moreover, it will be shown that these innovative technologies enable the recovery of valuable resources.

The implementation of wastewater treatment technology in many countries is far behind the required capacity. This is due to various reasons, including too costly treatment methods, and complicated technology. The use of algal technology, in its various forms, hopefully will help us to develop innovative technologies that are not too complicated or expensive to operate and maintain, while still producing good effluent qualities. I wish that the presentations and discussions of this seminar will be a step into that direction!

Peter van der Steen

Associate Professor of Environmental Technology

PRACTICAL INFORMATION

Venue for the seminar is auditorium A1B (first floor). Please note that drinks and food are not allowed in this (our newest) lecture room. Coffee breaks and lunch are in the restaurant (ground floor).

WIFI can be accessed through the network 'IHE-public' with password 'iheinternetkey'
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PROGRAMME

8.45 - 09.15 Registration

09.15 - 09.30 Introduction, *Algal technologies for wastewater treatment and resource recovery; application in developing countries*, Peter van der Steen, PhD, UNESCO-IHE Institute for Water Education, Delft, the Netherlands

09.30 - 10.15 Key-note, *Integration of microalgae and anaerobic digestion within a biorefinery approach for reuse and/or recovery of human residues*. Dr. Jean-Philippe Steyer, INRA, Laboratoire de Biotechnologie de l'Environnement, Narbonne, France

10.15 - 10.45 Coffee Break

State-of-the-art: applications at semi-full scale

10.45 - 11.15 *A novel fixed film algae based wastewater treatment system*, Udi Leshem, CEO, Aquanos, Israel

11.15 - 11.45 *Recovery of nutrients from concentrated wastewaters by microalgae*, Dr. ir. Tania V. Fernandes, NIOO-KNAW (Netherlands Institute of Ecology), the Netherlands

11.45 - 12.15 *A cost-benefit analysis for the processing of different waste sources, with relation to algal biomass productivity, biochemistry and potential value*, Dr Alla Silkina, Swansea University, UK

12.15 - 12.45 *Microalgae Technology for Urine Treatment*. Kanjana Tuantet, MSc, Wageningen University & Research Centre, the Netherlands

12.45 - 14.00 Lunch
Algal-bacterial systems for wastewater treatment

14.00 - 14.30  Organic matter and nutrients removal in a high rate pond using a microalgae-bacteria system: Influence of hydraulic retention time, Prof. German Buitron, Laboratory for Research on Advanced Processes for Water Treatment. Unidad Academica Juriquilla, Instituto de Ingenieria, Universidad Nacional Autonoma de Mexico, Mexico

14.30 - 15.00  The truth about MaB-flocs: features, challenges and outlook, Dr. ir. Sofie Van Den Hende, INTERREG IVB NWE project EnAlgae, Laboratory of Industrial Water and Ecotechnology (LIWET), Ghent University, Belgium

15.00 - 15.30  Comparing nitrogen removal by microalgae-bacteria consortia with algae consortia in a sequencing batch reactor, Angelica Rada MSc, UNESCO-IHE Institute for Water Education, the Netherlands

15.30 - 16.00  Coffee Break

Potential algae-based resource recovery; nutrients and biofuels

16.00 - 16.30  The application of microalgae as a slow-release fertilizer: tomato cultivation as a model Eng. Joeri Coppens, Laboratory of Microbial Ecology and Technology (LabMET), Ghent University, Belgium.

16.30 - 17.00  Selective pressures in microalgae cultivation, Ir. Peter Mooij, Department of Biotechnology, Delft University of Technology, the Netherlands

17.00 - 17.15  Closing remarks, Peter van der Steen, PhD, UNESCO-IHE Institute for Water Education, Delft, the Netherlands
ABSTRACTS

INTEGRATION OF MICROALGAE AND ANAEROBIC DIGESTION WITHIN A BIOREFINERY APPROACH FOR REUSE AND/OR RECOVERY OF HUMAN RESIDUES

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In the energy and chemical sectors, alternative production chains should be considered in order to simultaneously reduce the dependence on oil and mitigate climate change. Biomass is probably the only viable alternative to fossil resources for production of liquid transportation fuels and chemicals since, besides fossils, it is one of the only available sources of carbon rich material on earth. Over recent years, interest towards microalgae biomass has grown in both fundamental and applied research fields. The biorefinery concept includes different technologies able to convert biomass into added value chemicals, products (food and feed) and biofuels (biodiesel, bioethanol, biohydrogen). As in oil refinery, a biorefinery aims at producing multiple products, maximizing the value derived from differences in biomass components, including microalgae. This presentation will provide an overview of the various microalgae-derived products, focusing on anaerobic digestion for conversion of microalgal biomass into methane. Special attention will be paid to the range of possible inputs for anaerobic digestion (microalgal biomass and microalgal residue after lipid extraction) and the outputs resulting from the process (e.g. biogas and digestate). The strong interest for microalgae anaerobic digestion lies in its ability to mineralize microalgae containing organic nitrogen and phosphorus, resulting in a flux of ammonium and phosphate that can then be used as substrate for growing microalgae or that can be further processed to produce fertilizers. At present, anaerobic digestion outputs can provide nutrients, CO2 and water to cultivate microalgae, which in turn, are used as substrate for methane and fertilizer generation.
A NOVEL FIXED FILM ALGAE BASED WASTEWATER TREATMENT SYSTEM
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CEO, Aquanos, Israel

Aquanos Ltd. has developed a unique wastewater treatment technology, which produces a high quality effluent while utilizing a fraction of the energy required by conventional wastewater treatment plants (WWTPs). The system is based on a novel approach, using microalgae to produce the oxygen required for aerobic wastewater treatment, which then takes place on a fixed-film system.

Coupling this energy-effective aerobic treatment system with anaerobic pretreatment system results in system which is energy-positive. In addition to energy generation, the proposed system utilizes the algae produced in the treatment process, as an instrument to harvest nutrients from the wastewater, which can be used to produce value algal products such as fertilizers, animal feed and bio plastics.

The single largest expenditure in modern wastewater treatment is the energy required for aeration. As an alternative to mechanical aeration, oxygen produced by microalgal photosynthesis can be utilized, thereby dramatically reducing the energy requirements and operational expenditures of WWTPs.

Transfer of oxygen from microalgae to the aerobic biological system is highly efficient as microalgae produce oxygen in dissolved form; dissolved oxygen produced photosynthetically can reach supersaturation concentrations (15-30 mg L⁻¹). Microalgae not only supply oxygen for the oxidation of organic matter, but also remove nutrients from the effluent. The nutrients are used by the microalgae to produce biomass that can be valorized for energy production or other products (e.g bio fertilizers, bio plastics etc.). The symbiotic relationship between algae and microorganisms had been used in extensive treatment processes (mainly lagoons and high rate algal ponds) for many years. However, while having a symbiotic relationship, high concentrations of Mixed Liquor suspended Solids prevent light from penetrating into the water and thus hinder algae growth. As a result, in traditional algae-based wastewater treatment systems such as oxidation ponds, a low concentration of biomass is retained and long retention times are required, resulting in large footprints.

The Aquanos process overcome these obstacles by dividing the process into 2 separate reactors:

a) Fixed-film biological treatment: in this stage of the biological treatment, the microorganisms are attached to a media, allowing for high concentration of microorganisms to be retained in a separate reactor and thus prevent shading and allow light to penetrate the algae ponds.

b) Algae growth area (Raceway pond): In these shallow, engineered algae ponds, the algae will utilize nutrients and CO2 to grow, while producing water which is super-saturated with photo-synthetically produced oxygen (more than 20 mg/L oxygen concentration in the algae ponds, in contrast to the 8-9 mg/L normal oxygen saturation in water). The oxygen-
saturated liquid produced in the algae ponds will then recirculated to the fixed-film reactor. Thus, the microorganisms in the fixed film reactor are receiving two streams, one of raw WW and another of oxygenated water from the raceway ponds.

The end result is high effluent quality in a fully engineered process without the usage of the mechanical aeration. The technology had been tested for over 3 years in two pilot plants in Israel and the plants had proved the ability of the Aquanos process to produce high quality effluent without the usage of any mechanical aeration and in relatively short hydraulic retention time (24 hrs.) in the algal ponds. First full scale applications are being currently built in Israel and the US.
Welcome to the new world! The world where we have learned from Nature how to live in a cyclic natural balance – just like all other species – the waste of one is the resource of the other.

Current wastewater treatment systems do not fulfill the ideology of waste being a resource, as they aim at Removal and not Recovery. Therefore we need to rethink the toilet, stop flushing our toilets with loads of drinking water and provide the right technologies to recover all the richness present in our excreta. One of these richnesses is phosphorus – a depleting resource within the coming century [1]. As without phosphorus our lives cease to exist, the need to find alternative sources of phosphorus is a societal priority. Of all domestic wastewater produced in a household, urine has 40% of phosphorus while faeces has 28% of phosphorus [2]. Therefore when able to recover that phosphorus, human excreta could supply 22% of the world phosphorus demand [3].

The Dutch Institute of Ecology (NIOO-KNAW) has implemented in its new building a new sanitation concept where black water (toilet water) and grey water (shower/washing water) are separated at source, therefore facilitating nutrient recovery, reducing drinking water consumption, generating bioenergy and a valuable algal biomass product. The black water (BW) is treated in an UASB (upflow anaerobic sludge blanket) reactor and the remaining effluent, which is rich in nutrients (N, P, K), is intended to be post-treated by an algae photobioreactor (PBR), which is currently under research.

The current research focuses on recovering the nutrients present in digested black water (DBW = effluent of UASB reactor) by microalgae. Screening experiments have been followed by controlled flat panel PBR experiments. The results show that green alga, such as Chlorella vulgaris, Chlorella sorokiniana, Scenedesmus obliquous and Chlorella Pyrenoidosa successfully grow on DBW without any dilution of this concentrated medium (± 1 gNH₄⁺-N.L⁻¹ and ± 80 mgPO₄-P.L⁻¹). Biomass concentrations up to 12 g DW.L⁻¹ together with full nitrogen and phosphorus were reached for some of the species, therefore showing that nitrogen and phosphorus recovery by microalgae has a great potential for decentralised wastewater treatment [4].

References


A COST-BENEFIT ANALYSIS FOR THE PROCESSING OF DIFFERENT WASTE SOURCES, WITH RELATION TO ALGAL BIOMASS PRODUCTIVITY, BIOCHEMISTRY AND POTENTIAL VALUE
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The Water Framework Directive (WFD) was implemented in 2000, and aims to bring all inland water bodies to a standard denoted as having a ‘good ecological status’. Additionally the Habitats Directive requires water discharged into inland waters to have nutrients removed. This legislation makes it compulsory for waste producers to remediate their effluents, especially those in liquid form and are likely to cause significant ecological disturbance if discharged to the environment. More than 10 million tons of sludge wastes are produced in Europe, meaning these wastes are a substantial remediation problem. However they are also a potential source of nutrients from microalgal growth. These wastes contain high levels of the nutrients required for algal growth including Nitrogen and Phosphorous.

In this paper, different forms of liquid wastes have been investigated as a nutrient source for the production of algal biomass, processing costs were evaluated and a discussion was made as to whether a saleable product was generated. Waste sources including aquaculture waste, cattle waste and municipal waste were investigated in terms of their ability to produce algal biomass with particular valuable biochemical characteristics.

Liquid municipal waste, which is a bi-product of anaerobically digesting municipal waste from Welsh Water, was found to produce the greatest biomass density. The biomass also contained the highest average protein levels of 52.6%±2.7. This level of protein suggests the biomass could be successfully used as feed for Shrimp and other farmed crustaceans. Shrimp feed is typically sold for €11 per kg. As a comparison stuvite production, which is an emerging technology for the recovery of phosphate in the form of struvite fertiliser, has a value of approximately €0.08 per kg. The cost of producing this waste stream is currently borne by Welsh Water and is performed as standard on most municipal waste treatment plants. The only further processing that the waste underwent was membrane microfiltration through a 0.2 µm filter, which minimised the post processing cost. The cost of the microfiltration process was estimated to be €0.3 per m³. In order to produce 3kg of microalgae, 25L of processed waste is required demonstrating that the processing costs to produce nutrients for cultivation, from this form of waste, were minimal.

In contrast the cattle and fish farm waste was processed by acidifying to pH 3 to prevent volatization of Ammonia and to increase the Phosphorous recovered from solids in the sludge. It was then centrifuged, returned to pH 7 and filtered using membrane microfiltration. This process cost was €1.23 per m³.

The carbohydrate levels in the biomass produced using municipal waste were low compared to values found in the literature and lower than the biomass produced using the fish and cattle waste. This resulted in lower than anticipated biomethane levels, lower that that typically
produced from the industry standard of vegetable waste. However the methane levels were comparable to those produced using slurry and whey waste.

It was found that despite the reduced bioenergy potential the most economically viable waste source is anaerobically digested municipal waste as this requires minimal processing and minimal energy input and results in high algal growth rates and a useful high quality algal product.
Microalgae employ light, nutrients and CO$_2$ to grow and their biomass is useful for many applications for example production of chemicals, biodiesel, bioenergy, etc. These characteristics make microalgae a perfect tool to couple wastewater treatment with biomass production and also CO$_2$ sequestration. In domestic wastewater, the major source of nutrients comes from urine which can be separately collected and treated for more efficient nutrient recovery. Because urine is rich in nutrients it becomes a potential nutrient source for microalgae. The aims of this study were to optimize the productivity and nutrient recovery efficiency of microalgae grown in minimally diluted source-separated human urine under continuous illumination and under day/night cycle. A short optical-path (0.5 and 1.0 cm) photobioreactor was selected for cultivation of a selected species, *Chlorella sorokiniana*. Results of this study demonstrated high microalgae productivity and efficient nutrient recovery rate at optimum reactor dilution rates under continuous irradiance of ~1,500 mol-photon m$^{-2}$ s$^{-1}$. The highest biomass productivities with five times diluted synthetic and undiluted human urine respectively were 1.06 g-dw L$^{-1}$ h$^{-1}$ and 0.79 g-dw L$^{-1}$ h$^{-1}$. The respective biomass yields on light were 0.98 and 0.74 g-dw mol-photons$^{-1}$. Maximum nitrogen and phosphorus removal rates were as high as 120 mg-N L$^{-1}$ h$^{-1}$ and 19 mg-P L$^{-1}$ h$^{-1}$ with synthetic urine, and 89 mg-N L$^{-1}$ h$^{-1}$ and 8 mg-P L$^{-1}$ h$^{-1}$ with human urine. Simultaneously, in human urine COD was removed by a natural growing co-culture of aerobic biomass, with a maximal removal rate of 110-160 mg-COD L$^{-1}$ h$^{-1}$. Under day/night cycle the biomass yield on light decreased by 20%. The advantage of using microalgae for nutrient recovery from urine over other available recovery techniques like struvite precipitation is that it allows almost complete recovery of phosphorus together with a significant fraction of nitrogen (>50%). Furthermore, a substantial amount of COD (up to 75%) is simultaneously removed. The microalgae biomass can be applied as a slow-releasing fertilizer or supplied as a feedstock for production of biodiesel, chemicals or other energy carrier compounds. This study demonstrated the potential of concentrated urine treatment coupling with microalgae biomass production for areas with high irradiance.
In this study we evaluate the influence of the Hydraulic Retention Time (HRT) on carbon and nutrients removal with a microalgae-bacteria system as well as the biogas production with the biomass generated in the system. For that, a 50L high rate algal pond (HRAP) was used to study the influence of three different HRTs (2, 6 and 10 days) on nutrients removal, lipid productivity and methane potential of real domestic wastewater (after preliminary treatment). The reactor was operated under laboratory conditions and was first inoculated with a native microalgae consortium. It was observed a very good Chemical Oxygen Demand (>85%), ammonium (>97%) and phosphorous (up to 30%) independently of the HRT utilized. However, the mechanisms of NH$_4^+$ removal (assimilation and nitrification) were affected when the HRT decreased. The percentage of assimilation decreased with lower TRH. Also, morphological variations in the granules and the filamentous structures formed in the reactor were observed as the HRT decreased. High settling efficiency was observed due to agglomeration and granules as dominant structures through all the experimentation process. The chemical analysis of the biomass (macromolecular cell components) did not exhibit any change in both carbohydrates and lipids; nevertheless, their productivity showed an increasing trend with the decrease of HRT. Biochemical methane potential tests made evident the interest of using microalga-bacteria biomass to produce methane without pre-treat the biomass to disrupt the microalgae cell wall.
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Scientific studies on engineered, sunlight-powered microalgal bacterial systems for aerobic wastewater treatment based on photosynthetic aeration and biomass production dates back from over 40 years ago. Nevertheless, several challenges still hamper the implementation of these systems in Northwest Europe. A major challenge is the high cost of the separation of the treated wastewater from the microalgal bacterial biomass. To address this, the concept of microalgal bacterial flocs in sequencing batch reactors (MaB-floc SBRs) was developed. Operation as SBR selects fast-settling MaB-flocs via bioflocculation. This results in a costless separation of the MaB-flocs from the treated wastewater, akin to conventional activated sludge (CAS) systems. This concept was screened in lab-scale reactors for treatment of various wastewaters and was up-scaled to an outdoor raceway pond for treatment of aquaculture and food industry wastewater in Belgium.

The currently widely applied CAS systems for wastewater treatment based on mechanical aeration have been described in 1913, and are still being optimised one century later. Compared to CAS technology, the MaB-floc SBR technology is still very new. It is thus not a cliché to state that further optimisation of MaB-flocs SBRs is needed before it will be implemented on industrial scale. To identify the critical research needs for MaB-floc SBRs, an overview of the features and limitations of this system is presented. Bioflocculation, reactor operation, wastewater treatment, flue gas injection, biomass harvesting, biomass productivity, economics and environmental impact assessment, legislation and policy are discussed. This overview is based on the research outcomes obtained during 7 years doctoral and postdoctoral research, but also on hands-on-experience and valuable discussions with various stakeholders. Furthermore, future perspectives for the application of MaB-floc technology are outlined to set the stage for future collaborations.
COMPARING NITROGEN REMOVAL BY MICROALGAE-BACTERIA CONSORTIA WITH ALGAE CONSORTIA IN A SEQUENCING BATCH REACTOR
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Microalgae-bacteria consortia are considered as a novel option for wastewater biotreatment. Microalgae, through the photosynthetic process, are capable of assimilating CO₂, and release oxygen, which can be used by heterotrophic and autotrophic bacteria to oxidize organic matter and remove nutrients. In order to elucidate and compare the mechanisms of nitrogen removal, oxygen production and growth rate in microalgae-bacteria consortia, three reactors are currently operated in the laboratory of UNESCO-IHE. Each of the reactors was inoculated with a different microbial or algae population. Reactor 1 was inoculated with a mixture of algae and bacteria, reactor 2 with a mixture of 5 algae strains and reactor 3 with solely a strain of *Chlorella vulgaris*. The reactors were exposed to different hydraulic retention times and ammonium loading rates. The concentrations of nitrogenous compounds have been measured and physical parameters such as dissolved oxygen and pH have been recorded. Preliminary results have shown higher nitrogen removal efficiencies for reactor 1, with values up to 5.6 mgN/L/h of nitrogen removal, from which 3.9 mgN/L/h was removed through nitrification and the remaining through algae uptake. In contrast, in reactor 2 and 3 algae uptake was the main nitrogen removal mechanism, with maximum values of 4.4 and 4.6 mgN/L/h respectively. The differences in ammonium removal rates, and furthermore in the nitrogen algae uptake are the result of differences in the growth rate of algae and nitrifiers. Therefore, further studies and calculations are being conducted to elucidate accurately the specific growth rates in microalgae-bacteria systems. The results till date have proven that microalgae-bacteria consortia are capable of removing high concentrations of ammonium without external aeration and achieving low nutrient concentrations in the effluent.
THE APPLICATION OF MICROALGAE AS A SLOW-RELEASE FERTILIZER: TOMATO CULTIVATION AS A MODEL

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Nutrients such as nitrogen and phosphorus are key elements for plants and animals and are therefore essential for the food supply chain. Today nearly half of the global population is relying on mineral fertilizers for food production. The global challenges that face us, such as population growth and the evolution towards a more protein-rich diet, have as a consequence that the demand for agricultural produce continuously rises. This evolution puts pressure on the availability and prices of fertilizers. Sustainable nutrient management, including the recycling of nitrogen and phosphorus from human and agricultural waste streams, is therefore seen as a necessary factor to ensure global food security.

The use of microalgae has been described extensively within environmental biotechnology. Microalgae efficiently utilize and remove the nitrogen and phosphorus present in wastewater and assimilate these in valuable algal biomass. Depending on the supplied wastewater, the nutrient-rich algal biomass are subsequently valorised for feed purposes or as a feedstock for biorefineries. Yet, besides macronutrients such a N, P and K, microalgae also contain plant growth-promoting substances such as vitamins, carotenoids, amino acids and antifungal substances. In this study, the valorisation of microalgae as an organic slow-release fertilizer is presented.

The fertilizer potential of both fresh water and marine microalgal biomass was determined; more specifically dried microalgal bacterial flocs (MaB-flocs), cultivated on aquaculture wastewater, and unialgal *Nannochloropsis* biomass. In a first stage the mineralization rate of both types of biomass was determined. Also a dosage toxicity test for Ca²⁺ and Na⁺ was performed using seedlings of lettuce and garden cress, respectively. Subsequently, a greenhouse tomato growth experiment was performed, wherein the fertilizer potential of both types of microalgal biomass was compared with conventional inorganic and organic horticulture fertilizers. The growth rate of the tomato plants and the tomato yield were assessed for each fertilizer treatment, as well as the leaf composition and the water, sugar and carotenoid concentrations in the tomato fruits. The results of the study show there was no significant difference in plant growth between the fertilizer treatments with MaB-flocs, *Nannochloropsis* and the conventional organic fertilizer. Also higher sugar concentrations in the tomato fruits were obtained compared to the inorganic fertilizer. This demonstrates the potential of microalgae as a high-value slow-release fertilizer for horticulture applications.
Large-scale production of energetic storage compounds by microalgae is hampered by competition and evolution. Both phenomena result in loss of productivity and arise due to a mismatch between the desired characteristic and the imposed environment. The prevailing approach to solve this issue aims at increasing the survival potential of the desired strain, by for example working in closed systems or at extreme conditions. This creates however an economic burden and the issue of strain degeneration is not addressed by increasing the survival potential of the desired strain. We therefore advocate to focus on the imposed cultivation environment, rather than on the desired species. In any open system the environment selects for the most fitting organisms and this holds also true for microalgal cultivation system. If we aim for a certain characteristic – such as production of energetic storage compounds – we should design an environment in which displaying this characteristic is rewarded, by for example coupling it to an increased chance of survival.

We’ve shown that uncoupling of carbon fixation in the light and nutrient uptake in the dark enriches and sustains carbohydrate producing microalgae. These algae produce up to 57% carbohydrates on organic weight in the nutrient limited light phase, and consume these partly to produce biomass in the nutrient excess dark period. Both the community structure as the functional characteristics of these enriched microalgal communities are highly stable in time in open systems.

With a clear list of desired traits and an overwhelming microalgal diversity in nature, we encourage the application of ecology based selective environments in microalgal cultivation.