Method and apparatus for use in the treatment of water

Field of Invention

This invention relates to a method and apparatus for use in the treatment of water. In particular, it relates to an improved method and apparatus for use in the treatment of wastewater.

Background of the Invention

The listing or discussion of a prior-published document in this specification should not necessarily be taken as an acknowledgement that the document is part of the state of the art or is common general knowledge.

Current state-of-the-art water treatment systems generally include the following phases.

i. A Preliminary Treatment phase that remove large solids (e.g. trash, tree limbs, sand, glass, large agglomerations of paper or other solid materials like fibre) from the wastewater to avoid clogging or damage to the apparatus. This is usually achieved using bars or a grill in combination with a manual or automatic rake to prevent the solids from blocking the bars or grill.

ii. A Primary Treatment phase, where the wastewater is allowed to separate into phases for separate treatment. These phases are generally solid phases (i.e. sludge) and liquid phases (i.e. clarified wastewater, grease and oils).

iii. A Secondary Treatment phase that substantially degrades the content of each phase into less harmful substances, sometimes with further phase separation and/or extraction of useful substances (e.g. biogas, fertilizer).

iv. A Tertiary Treatment phase that improves the effluent quality to meet standards for discharge from the water treatment system (e.g. into the environment, storage or further processing).

Each of the above phases can comprise one or more treatment processes. For example, the Secondary Treatment phase can comprise aerobic and/or anaerobic processes to degrade the dissolved or suspended substances in the wastewater.

An example of an aerobic process is the activated sludge process, where certain microorganisms are mixed into wastewater that has undergone the Primary Treatment phase. These microorganisms help oxidise carbonaceous or nitrogenous biological matter, remove phosphates, and flocculate and settle suspended and dissolved substances out of the liquid phase. Air or oxygen is added to the wastewater to allow the microorganisms to grow and function quickly. Therefore, at the end of the process the treated wastewater has lower levels of dissolved and suspended material (i.e. pollutants).

One parameter to evaluate the activated sludge process is the Food to Mass ratio (F:M ratio). F:M describes the relationship between the load (i.e. kg/day as opposed to mg/L) of Biological Oxygen Demand (BOD, or bacterial 'food') entering the aeration plant and the 'mass' of bacteria in the aeration tank available to treat the incoming BOD. F:M ratio reflects the capability of a treatment process in terms of the pollutant load imposed on (and hence substrate availability to) the microbial population. A defined F:M ratio may result in certain microbial communities dominating as the substrate availability condition shifts the microbial population towards conditions between growth and decay. The latter conditions have impact on aeration requirements and excess sludge generation. For example, F:M conditions promoting decay may increase aeration requirements and result in larger amounts of sludge being generated.

The F:M ratio may also be measured in terms of Chemical Oxygen Demand (COD). This is subject to an appropriate conversion factor depending on the substrate, e.g. for municipal wastewater this conversion factor may be reflected in a COD:BOD ratio in the range of from about 2:1 to about 6:1.

Contact stabilization is an activated sludge variant which returns microbial biomass to the front of the aeration basin to facilitate sorption of incoming soluble organic pollutants for subsequent aerobic degradation. This mode of operation allows for more rapid removal of carbonaceous pollutants from the liquid phase and is primarily dependent on the surface characteristics of the microbial cells in the biomass so returned. The return biomass is typically from the secondary clarifier of the wastewater treatment plant. While contact stabilization does enhance the speed with which organic pollutants are removed, it does not necessarily reduce oxidation (and hence) aeration requirements during the subsequent stage. Energy consumption in relation to bio-oxidation is therefore not necessarily substantially different from a conventional activated sludge process. There are also limits as to how much of the incoming soluble carbonaceous pollutants can be removed in this

manner (typically 20%). Sorption in this instance is driven more by adsorption and so by the concentration gradients created by the flow arrangement.

The biomass remains very much the facultative microbial population cultivated in the activated sludge process and the surface characteristics of the microbial population have not been intentionally manipulated to enhance adsorption other than, in some instances, to reduce the food to microorganism (F/M) ratio.

Since 2008, Siemens has described their research on a somewhat similar approach with the idea of adsorbing the incoming carbonaceous pollutants with activated sludge biomass. Biosorption performance has been tested on live and dead biomass. A major departure from the contact stabilization approach in this instance is that instead of allowing the biomass which has sorbed the carbonaceous pollutants to continue into the activated sludge aeration basin, it is removed and channeled to an anaerobic digester. The former action reduces oxidation requirements at the aeration basin (and so reduces the energy requirements) while the latter results in biogas and hence energy recovery.

On the basis of available information on the Siemens method, it does not appear the biomass (other than the dead option) has been modified to enhance its sorption capacity other than with what is known to apply for contact stabilization. This means that the biomass sorption performance is unlikely to be superior to the performance of the biomass used in a conventional contact stabilization process and therefore remains essentially driven by adsorption.

Contact stabilization and the Siemens methods are believed to be primarily driven by surface adsorption if they are applied after primary clarification and with biomass returned from the secondary clarifier. Alternatively, these methods are believed to operate through the entrapment by the enmeshment mechanism, coupled with surface adsorption when applied without primary clarification. The enmeshment mechanism requires the use of biomass that has a filamentous morphology to trap pollutants. However, both of these methods typically seek to minimize the amount of filamentous biomass. This is because this biomass morphology may lead to subsequent difficulties in anaerobic digestion and sludge dewatering.

Given the above, biosorption performance still remains low and the wastewater treated by these methods retains a high carbon content, resulting in large downstream aerobic oxidation energy requirements. Further, the increase in biogas and energy recovery is

relatively minor. Therefore, there remains a need for improved systems and methods that can improve the treatment of wastewater and in particular by way recovering the wastewater's carbon content and so reduce energy for treatment requirements.

Summary of Invention

This invention relates to a method and apparatus for use in the treatment of water. In particular, it relates to an improved method and apparatus for use in the treatment of wastewater. This method may enable various combinations of reduced energy requirements and/or increased energy recovery by emphasizing various combinations of bioaccumulation, entrapment and/or surface adsorption of substances such that these substances are removed from the water being treated.

In a first aspect of the invention, there is provided a method of treating wastewater, comprising the steps of:

(i) culturing a first and/or a second and/or a third microbe population in a first, a second, and a third vessel, respectively, the first microbe population being capable of bioaccumulating at least one pollutant, the second microbe population being capable of producing a high level of Extracellular Polymeric Substances (EPS) and/or being capable of entrapment of at least one pollutant, and the third microbe population being capable of surface adsorption of at least one pollutant;

(ii) dividing the cultured first and/or second and/or third microbe populations into a first portion and at least a second portion, respectively, wherein each respective at least second portion is capable of regenerating the respective microbe population;

(iii) contacting a main stream of wastewater comprising at least one pollutant with the first portion of the first and/or second and/or third microbe populations, such that the first portion of the first and/or second and/or third microbe population removes at least part of the at least one pollutant from the wastewater; and

(iv) removing at least part of the first portion of the first and/or second and/or third microbe populations from the main stream of wastewater.

Embodiments of the first aspect are set out in Claims 2 to 30.

A second aspect of the invention relates to an apparatus for wastewater treatment, comprising one or more culturing vessels for culturing microbe populations, said culturing

vessels being in fluid communication with at least one contact vessel for contacting portions of cultured microbe populations with a main stream of wastewater, wherein the at least one contact vessel is in fluid communication with the main stream of wastewater. Embodiments of this aspect are set out in Claims 32 to 37.

Description of Figures

Fig. 1 Biosorption capacity in terms of the 3 mechanisms for biomass S1 and S2 under different pH conditions

Fig. 2 Comparison of Bioaccumulation (uptake) and surface sorption performance of biomass S1 and S2 under different pH conditions

Fig. 3 Pictures of PHA staining for biomass S1 and S2 under pH=7 and pH=8

Fig. 4 Calorific value of biomass S1 and S2 before and after biosorption under different pH conditions (contact time = 10 mins)

Fig. 5 Optical microscope image (x10, x20) of biomass D1 and D2

Fig. 6 Biosorption capacity in terms of the 3 mechanisms for biomass D1 and D2 under different pH conditions

Fig. 7 Comparison of Surface sorption and Bioaccumulation performance of biomass D1 and D2 under different pH conditions

Description

The invention will now be described in further detail below.

It is postulated that biomass sorption of pollutants can involve at least three mechanisms – surface adsorption, entrapment, and carbon uptake (C-uptake).

Surface adsorption is relevant with respect to soluble carbonaceous pollutants. It involves the transfer of solutes from the liquid phase onto the surface of the sorbent (i.e. the microbial cell – dead or live). The capacity (or available surface area) of the microbial cell to hold the

sorbate is dependent on (among other factors) the manner with which the microbial cells have been prepared, which affects its surface characteristics.

Entrapment can occur because of the morphology of the biomass applied (i.e. filamentous and so entrapment by enmeshment) and/or because of the presence of Extracellular Polymeric Substances (EPS, a microbial cell secreted "sticky" substance which enhances agglomeration of particulates and so entrapment by adhesion).

Generation of EPS by biomass and its application can only be applied as a purposeful method if the microbial community and its activities can be manipulated. While it has been reported which microbes and under which circumstances microbes are capable of producing EPS, these reports have not yet been adequately translated into an engineered approach towards effective use of the entrapment technique. That is, entrapment by adhesion, in water treatment. Entrapment by enmeshment is also not typically used due to associated problems with dewatering and anaerobic digestion. However, entrapment by enmeshment is, in some cases, desirable for certain pollutants and the associated problems can be mitigated by suitable preparation of the microbial population. The present method allows for culture and bio-augmenting of microbe populations with enhanced EPS generation capabilities that can facilitate entrapment by adhesion. Further embodiments relate to the culturing and bio-augmentation of microbe populations with suitable morphology for entrapment of pollutants by enmeshment.

The third mechanism is one of microbial uptake, or bioaccumulation and so requires live organisms. This mechanism again relates to the carbonaceous soluble (or solubilized) component. However, uptake sufficient only for (aerobic or anaerobic) metabolism would not result in a phenomenon that can be translated into a useful process for energy reduction and recovery. Microbial uptake (or bioaccumulation) has to be in excess of normal metabolic requirements in order to be more useful in removing pollutants from wastewater. In other words, the bioaccumulation of the pollutants by microbes must be a form of "luxury" uptake and accumulation (e.g. luxury carbon uptake). This requires identification of specific microbes and conditions for their culture so that an engineered bio-accumulation approach can be developed.

As used herein, "pollutant" refers to carbonaceous and other undesirable substances present in the wastewater, such as biological, organic and other waste matter. These substances are undesirable as they would cause problems if present in high levels in the wastewater that is discharged into the environment or discharged from the wastewater

treatment system. In the case of municipal wastewater, the pollutants may include dissolved and suspended waste matter such as fecal matter, organic acids, fibres and the like. In other types of wastewater such as industrial wastewater, the pollutants may include specific by-products or waste products from the industrial process in question, for example fine paper fibres. In some cases wastewater may comprise pollutants which are inorganic, e.g. metals and/or salts thereof. As the person skilled in the art would appreciate, the present invention is applicable to a range of wastewater types and the meaning of "pollutants" will therefore vary contextually.

As used herein, "biomass" and "microbial population" are used interchangeably and refer to the microbial populations contacted with the wastewater in the present invention. The microbial populations (and/or their cellular products) are able to remove at least part of the biological and waste matter from the wastewater by biosorption (also referred to as biomass sorption) via at least the three mechanisms discussed below. After an appropriate amount of time allowed for sorption, at least part of the microbial populations (and/or their cellular products) are separated from the wastewater, resulting in wastewater with less biological and waste matter than was present before the contacting step. Separation may be by phase separation, membrane, settling or any suitable method that allows the at least part of the biomass to be separated from the bulk of the wastewater.

After sorption of the pollutants from the wastewater, the biomass is separated from the bulk of the wastewater and directed to secondary processing, which includes anaerobic digestion. Anaerobic digestion results in the generation of biogas and therefore energy recovery.

The wastewater is also directed to secondary processing, which includes aerobic digestion. Since its carbon content has been reduced by the removal of carbonaceous pollutants by sorption into/onto the biomass, the subsequent treatment of the wastewater is reduced. Therefore, reducing the energy costs associated with its treatment.

In embodiments described herein, the identification and culture of microbes capable of luxury C-uptake, the Glycogen Accumulating Organisms (GAOs), has been accomplished. The subsequent bio-augmenting of this culture into the contact vessel for enhanced bio-accumulation of soluble carbonaceous pollutants has also been accomplished.

It is hypothesized herein that biosorption comprises three different mechanisms: surface sorption; entrapment as well as bioaccumulation. Lab studies have been executed to

investigate the performance for each of the above mentioned mechanisms from biomass harvested under different environmental conditions.

Previous methods (i.e. contact stabilization and Siemens) have essentially focused on only one of the three sorption mechanisms – surface adsorption. In contrast the method described in the embodiments below allows for deployment of all three – surface adsorption, C-uptake, and entrapment. In other words, the presently described method goes beyond using available biomass from the secondary clarifier or activated sludge for biosorption of pollutants, and further provides an improved approach that allows for deployment of all three sorption mechanisms – surface adsorption, C-uptake, and entrapment. This is achieved by using one or more microbial populations that are selectively cultured and bio-augmented to enhance sorption performance. This may be achieved using a single microbial population, or more than one microbial population. In this context, it should be noted that the use of, for example, microbes that primarily target bioaccumulation of a wastewater pollutant does not exclude the same microbes from also acting *via* surface adsorption and/or entrapment too. For example, a single microbial population may be cultured to enhance one or more of the three sorption mechanisms. However, deployment of one or more of the three sorption mechanisms may also be achieved using two or more microbial populations. Each microbial population may be cultured to enhance one or more sorption mechanisms. In some embodiments two or more microbial populations utilizing two or more of the mechanisms described above can be employed at the same time.

"Culturing" and "Bio-augmentation" as used herein may refer to any method of culturing, treatment and/or modification of a microbial population to have improved characteristics such as enhanced sorption performance (e.g. capacity, speed, selectivity for specific pollutants and/or improved calorific increase and/or more desirable by-products as a result of sorption of pollutants from wastewater). Such culturing, treatment and/or modification includes culturing on media optimized for specific strains or populations of microbes, culturing on media that discourages growth of undesirable strains or populations of microbes. It also includes preparing the biomass generated by the culturing of microbial populations, by treatment of the biomass with heat, chemicals, mechanical processes and the like to improve its characteristics. This may result in effects such as the release of more EPS. It may also result in the killing or inactivation of microbial populations in whole or in part, which may be beneficial to avoid or reduce further growth of these microbes in the wastewater being treated, thereby mitigating problems downstream of the present method. Culturing, treatment and/or modification includes isolation and identification of certain microbial strains and/or populations for selective culturing, and/or genetic modification of

microbes and/or microbial populations by any genetic modification and/or artificial selection technique to improve any of the above characteristics and thereby improve their sorption performance.

The developed methodology is described in more detail below.

a. The technology comprises two major components – the culture tank and the sorption tank. The culture tank shall typically be operated in a side stream mode with either the incoming wastewater or a formulated feed stream. The sorption tank shall typically be inserted into the treatment train of a wastewater treatment facility and sited just before the aeration vessel. Using sewage as an example, this technology can be inserted between the present preliminary (or primary) unit treatment processes and any aerobic process (e.g. activated sludge, MBR, etc). As the sewage passes the preliminary (or primary) unit processes, it is split into two streams. The larger stream goes to the contact tank while the smaller stream goes to the side stream culture tank. Microbes cultured in the culture tank are harvested and transferred into the contact tank. After sorption the pretreated sewage continues to any aerobic process while the biomass which has sorbed quantities of organics from the sewage and which has undergone liquid-solids separation is channeled into an anaerobic process.

b. The culture vessel (or vessels) allows for preparation of biomass which primarily targets some combination of bioaccumulation, surface adsorption, and binding with EPS.

c. The number of culture tanks present will be directly determined by the types of microbes that are to be used in treating the waste water. In general, there is one culture tank for each type of biomass. For example, microbes primarily targeting bioaccumulation are cultured in a separate tank to microbes primarily targeting surface adsorption, both of which are cultured in separate tanks to microbes primarily targeting the production of EPS.

d. Where bioaccumulation is emphasized, Glycogen Accumulating Organisms (GAOs) are cultured under conditions which may include the following – (a) anaerobic and aerobic phase alternation, (b) feed at the anaerobic phase, (c) dissolved oxygen control, (d) pH control, (e) temperatures between 25 and 45°C (e.g. a tropical climate). The identified GAO populations are mainly

Gammaproteobacteria GAOs (e.g. *Candidatus* Competibacter phosphatis), and *Alphaproteobacteria* GAOs (e.g. *Defluviicoccus* vanus-related organisms).

e. Where entrapment by adhesion is emphasized, the EPS producing microbes are isolated and cultured under conditions that include the following – (a) strains are isolated from a culture that has a high EPS content, (b) isolated strains are grown in nutrient enriched medium, (c) medium is optimized to each individual strain, (d) EPS or strains can be harvested and dosed into entrapment system. Identified EPS producing strains are *Pseudomonas sp., Bacillus sp., Pantoea sp., Serratia sp., Yersinia sp., Microbacterium sp., Enterobacter sp., Photorhabdus sp.* Where entrapment by enmeshment is emphasized, microbial strains and culture conditions favoring straight-chain microbial morphology are involved. The culture condition for this type of microbes should be low F:M ratios.

f. Where surface adsorption is emphasized, the microbial mass is cultured under conditions which may include the following – (a) low F/M, (b) nutrient deficiency, (c) low DO, (d) readily-metalobolizeable substrates (eg low MW organic acid, simple sugars), (e) high SRT, (f) presence of hydrogen sulfide. Identified microbes with large surface area are *Thiothrix sp.,* filamentous bacteria Type 0914, 0411 (*Flexibacter* subgroup of *Flexibacter-Cytophaga-Bacteriodetes phylum*), and 0961, *Nocardioforms, Nostocoida Limicola II and III, etc.* It has been reported that *Nocardioforms,* filamentous bacteria Type 0914 and 0411 will not cause sludge bulking.

g. The sorption tank can possibly be sequenced controlled (in which case it will serve as a contact and liquid-solids separation vessel on a temporal basis) or can use the existing PSTs for liquid-solids separation (if it follows the preliminary unit processes and is operated in a continuous flow mode) with the liquid stream going for polishing by the aerobic process and the solids stream going for anaerobic digestion and energy recovery.

As described herein, embodiments relate to a method of treating wastewater, comprising the steps of:

(i) culturing a first and/or a second and/or a third microbe population in a first, a second, and a third vessel, respectively, the first microbe population being capable of bioaccumulating at least one pollutant, the second microbe population being capable of producing a high level of Extracellular Polymeric Substances (EPS) and/or being capable of entrapment of at least one pollutant, and the third microbe population being capable of surface adsorption of at least one pollutant;

(ii) dividing the cultured first and/or second and/or third microbe populations into a first portion and at least a second portion, respectively, wherein each respective at least second portion is capable of regenerating the respective microbe population;

(iii) contacting a main stream of wastewater comprising at least one pollutant with the first portion of the first and/or second and/or third microbe populations, such that the first portion of the first and/or second and/or third microbe population removes at least part of the at least one pollutant from the wastewater; and

(iv) removing at least part of the first portion of the first and/or second and/or third microbe populations from the main stream of wastewater.

When used herein, "bioaccumulation" refers to the uptake of pollutants by the microbial population in question. For example, C-uptake.

In the above method, the use of the respective first portions of the first and/or second and/or third microbe populations in step (iii) may reduce the population sizes of the respective microbe populations remaining in the respective culturing vessels. Accordingly, when used herein, "regenerating" refers to the ability of each respective second portion of the first and/or second and/or third microbe populations to be cultured such that the respective microbe populations remaining in the respective culturing vessels return to a size that allows for the method to be repeated if necessary. For example, enough of each respective microbe population must be kept in the at least a second portion to keep the microbe population in the culture vessel viable and able to grow, while retaining the desirable characteristics that allow enhanced biosorption performance. This also applies when the microbe populations comprise more than one strain or species of microbe in a community.

In the above method, the steps (i) to (iv) can be repeated continuously in a sequential batch or a continuous flow process. In embodiments, the contacting step above lasts for at least

about 10 minutes. In some embodiments, the second microbe population is present and the first portion of the second microbe population comprises EPS. Preferably, the removed microbe population(s) and/or EPS are anaerobically digested. As discussed above, the culturing of the microbe community(ies) is within a first, a second and/or a third vessel that is in fluid contact with a side-stream of wastewater diverted from the main stream of wastewater comprising the at least one pollutant.

In some embodiments of the above method, the second microbe population is present and in step (ii) EPS is isolated from at least part of the first portion of the second microbe population; in step (iii) the EPS is contacted with the main stream of wastewater to remove the at least one pollutant; and in step (iv) at least part of the EPS is removed from the main stream of wastewater. When contacted to the main stream of wastewater the concentration of the EPS may be at least about 150mgEPS/gVSS in the culture vessel.

In some embodiments, the second microbe population is present and in step (ii) the EPS is not isolated and in step (iii) the EPS together with the first portion of the second microbe population is contacted with the main stream of waste water to remove the at least one pollutant and in step (iv) at least part of the EPS and at least part of the first portion of the second microbe population are removed from the main stream of wastewater.

The first microbe population being capable of bioaccumulating at least one pollutant selected from the group consisting of colloidal or/and soluble organic carbon pollutants. The second microbe population produces EPS capable of removing at least one pollutant selected from the group consisting of colloidal or/and particulate organic carbon pollutants, and/or the second microbe population is capable of removing by entrapment (enmeshment mechanism) at least one pollutant selected from the group consisting of particulate organic carbon pollutants. The third microbe population is capable of removing by surface adsorption at least one pollutant selected from the group consisting of colloidal or/and soluble organic carbon pollutants.

In the contacting step of the above method, the presence of the first microbe population in the main stream of wastewater is in the range of from about 0.3 to about 3 g/L. When contacted to the main stream of wastewater the concentration of the second microbe population (EPS producing microbes) is in the range of from about 0.3 to about 3 g/L. When contacted to the main stream of wastewater the concentration of the third microbe population is in the range of from about 0.5 to about 3 g/L.

When used in the method described above, the first microbe population is cultured under conditions that promote dominance of microorganisms capable of glycogen accumulation. For example, the first microbe population using at least one of: alternating between anaerobic and aerobic phases; providing feed in the anaerobic phase (e.g. where feed is provided during the anaerobic phase in an amount that that thereafter contributes to controlling dissolved oxygen levels, for example maintaining dissolved oxygen levels at between about 1.5 to about 2.5mg/L during the aerobic phase and/or maintaining dissolved oxygen levels at about zero during the anaerobic phase); controlling pH levels (e.g. maintaining pH levels at from about 6 to about 9, such as from about 6 to about 8.5, such as from about 7 to about 8.5, e.g. from about 7.5 to about 8.5); controlling the F:M ratio (feed:biomass) (e.g. maintaining the F:M ratio at from about 0.1 to about 0.3, such as about 0.15); and controlling temperature levels (e.g. maintaining temperature levels at from about 25 °C to about 45 °C).

Anaerobic and aerobic phases refer to phases in which no oxygen, and some oxygen, respectively, is supplied to the microbe populations under culture. Dissolved oxygen levels may change gradually during these phases as oxygen is used up by the growing microbes. In this context, maintaining dissolved oxygen levels at about zero during the anaerobic phase is to be understood as supplying no oxygen to the microbe population under culture and allowing the dissolved oxygen level to drop to about zero, for example below detection levels of standard dissolved oxygen sensors used in wastewater treatment methods.

In embodiments, the first microbe population, when present, comprises at least one microorganism that is capable of glycogen accumulation (e.g. comprising at least one microorganism of the class *Gammaproteobacteria* (e.g. *Candidatus Competibacter*) or *Alphaproteobacteria* (e.g. a *Defluviicoccus vanus*-related organism))

When the first microbe population is used in the method, it can comprise at least one microorganism with: a biosorption capacity of at least about 40 mg COD/g SS (Chemical Oxygen Demand per gram of biomass) (e.g. at least about 55 mg COD/g); and/or a bioaccumulation capacity of at least about 20 mg COD/g SS (e.g. at least about 35 mg COD/g SS); and/or a biomass calorific value increase of at least about 0.5 kJ/g SS (kilojoules per gram of biomass) (e.g. at least about 0.9 kJ/ g SS).

In embodiments where the second microbe population is used, it can comprise at least one microorganism isolated from a culture of microbes with high EPS content, in other words a culture of microbes having a high level of EPS. Said microorganism can be further cultured

in nutrient enriched media to obtain the second microbe population (e.g. said least one microorganism isolated from a culture of microbes with high EPS content is further identified and cultured in media that is optimized for its growth to obtain the second microbe population). For example, the second microbe population, when present, comprises at least one species selected from the group consisting of *Pseudomonas sp., Bacillus sp., Pantoea sp., Serratia sp., Yersinia sp., Microbacterium sp., Enterobacter sp., Photorhabdus sp.*

In embodiments, "a high level of EPS" can be a concentration of EPS of at least about 150mgEPS/gVSS.

In some embodiments where the second microbe population is used, it can comprise at least one microorganism isolated from a culture of microbes with cellular morphology suitable for entrapment of at least one pollutant by enmeshment. For example, a culture of microbes with cellular morphology suitable for entrapment of at least one pollutant by enmeshment may be cultured at low F:M ratio (e.g. from about 0.1 to about 0.3). At least one microorganism may be isolated from a culture of microbes with cellular morphology suitable for entrapment of at least one pollutant by enmeshment and further cultured in nutrient enriched media to obtain the second microbe population (e.g. the at least one microorganism isolated from a culture of microbes with cellular morphology suitable for entrapment of at least one pollutant by enmeshment is further identified and cultured in media that is optimized for its growth to obtain the second microbe population.). For example, the second microbe population, when present, comprises at least one species selected from the group consisting of *Pseudomonas sp., Bacillus sp., Pantoea sp., Serratia sp., Yersinia sp., Microbacterium sp., Enterobacter sp., Photorhabdus sp.* The media used to culture the at least one isolated microorganism may have a low F:M ratio, e.g. from about 0.1 to about 0.3. In some embodiments the second microbe population is divided into a first and at least a second portion, and the first portion is treated to prevent or reduce further growth, before being used to contact the wastewater.

When used in the method described above, the third microbe population is cultured using at least one of: low F/M (the F:M ratio at between about 0.1 to about 0.3, such as maintaining the F:M ratio at about 0.15); nutrient deficiency (e.g. a nutrient deficiency in the third vessel of: nitrogen concentration is less than 10mg/L and phosphate concentration is less than 1 mg/L); low dissolved oxygen levels (a dissolved oxygen level of from about 0.1-1.5mg/L); readily-metabolizeable substrates (e.g. a level of readily-metabolizeable substrates of about 150mg/L, where the readily-metabolizeable substrates are selected from the group consisting of low molecular weight organic acids and simple sugars.); high Sludge Retention

Time (SRT) (e.g. a SRT of at least about 50-60 days); and in the presence of hydrogen sulphide.

In some other embodiments, when used in the method described above, the third microbe population is cultured using at least one of: high F/M (the F:M ratio at between about 0.4 to about 0.6, such as maintaining the F:M ratio at about 0.45

When used in the method described above, the third microbe population may comprise at least one species selected from the group consisting of *Thiothrix sp., filamentous bacteria Type 0914, filamentous bacteria Type 0411 (Flexibacter subgroup of Flexibacter-Cytophaga-Bacteriodetes phylum), and 0961, Nocardioforms, Nostocoida Limicola II and III.*

In some embodiments, the presently disclosed method further comprises treating the respective first portions of one or more of the first, second and/or third microbe populations in step (ii), thereby improving their characteristics. The treating may use heat, chemicals, mechanical processes and the like, and/or combinations thereof, to improve characteristics of the respective first portions of one or more of the first, second and/or third microbe populations. This may result in effects such as improved biosorption, for example through the release of more EPS. It may also result in the killing or inactivation of the respective first portions of one or more of the first, second and/or third microbe populations in whole or in part, which may be beneficial to avoid or reduce further growth of these microbes in the wastewater being treated, thereby mitigating any potential problems caused by their growth downstream of the present method.

For example, in embodiments wherein the second microbe population is present, step (ii) may further comprise treating the first portion of the second microbe population, such that the treated first portion of the second microbe population has reduced or no further growth after contacting the main stream of wastewater.

In particular embodiments, the method above uses at least the first microbial population that is capable of bioaccumulation. For example, in particular embodiments:

step (i) comprises culturing the first microbe population in the first vessel, the first microbe population being capable of bioaccumulating at least one pollutant;

step (ii) comprises dividing the cultured first microbe population into a first portion and at least a second portion, wherein the at least second portion of the first microbe population is capable of regenerating the first microbe population;

step (iii) comprises contacting the main stream of wastewater comprising at least one pollutant with the first portion of the first microbe population, such that the first portion of the first microbe population removes at least part of the at least one pollutant from the wastewater; and

step (iv) comprises removing at least part of the first portion of the first microbe population from the main stream of wastewater.

Embodiments also relate to an apparatus for wastewater treatment, comprising one or more culturing vessels for culturing microbe populations, said culturing vessels being in fluid communication with at least one contact vessel for contacting portions of cultured microbe populations with a main stream of wastewater, wherein the at least one contact vessel is in fluid communication with the main stream of wastewater. For example, at least one of the one or more culturing vessels is in fluid communication with a side-stream of wastewater diverted from the main stream of wastewater.

In the above apparatus, at least one of the one or more culturing vessels is adapted to: control dissolved oxygen levels; and/or provide feed; and/or control the F:M ratio (feed:mass); and/or control pH levels; and/or control Sludge Retention Time (SRT); and/or control temperature levels; and/or control hydrogen sulphide levels. In embodiments, the apparatus is adapted to run a sequential batch or continuous flow process to treat the wastewater.

In general, the at least one contact vessel is arranged upstream of an aerobic treatment vessel adapted to receive the wastewater. For example, the at least one contact vessel is arranged upstream of an anaerobic treatment vessel adapted to receive at least one portion of cultured microbe population that has been contacted with the main stream of wastewater. In some embodiments, the at least one contact vessel is configurable as a primary settling tank for liquid-solid separation of the main stream of wastewater.

While the method provides for the use of one or more (e.g. two or more) microbe populations cultured separately in culturing vessels. These populations may be entirely distinct or may comprise one or more common strains of bacteria, but are each cultured in such a way as to emphasize different aspects of biosorption. For example, microbe populations may be cultured to allow dominance of species and/or communities that have increased capacities and tendencies for bioaccumulation of pollutants (i.e. by luxury uptake of the pollutant as a nutrient, beyond metabolic requirements). Other microbe populations may be cultured to enhance entrapment by allowing dominance of species/populations suited to entrapment of

the desired pollutant, e.g. species with filamentous cell morphology suitable for entrapment by enmeshment, and/or species with increased excretion of extracellular polymeric substances (EPS) suitable for entrapment by adhesion. As microbe populations comprising the same dominant species may in some instances exhibit different morphologies such as filamentous cell structures, EPS secretion or both, in some embodiments one or more microbe populations may comprise one or more of the same dominant species. Yet other microbe populations may be cultured to allow the dominance of species with a greater or more suitable cellular surface area for surface adsorption of pollutants. However, while the above culturing conditions may place the emphasis on one or more modes of biosorption, it does not exclude the possibility of each microbe population exhibiting some of the other modes of biosorption, as may be seen in the examples and figures. Accordingly, a microbe population cultured to have maximal bioaccumulation may still exhibit significant surface adsorption. This usefully increases the amount of biosorption of pollutants and the speed at which the pollutants are removed from the wastewater.

The method, apparatus and microbial cultures shall have application where there is interest in wastewater treatment with energy reduction and recovery. The latter is of growing importance given the growing awareness of the energy-environment nexus. Current state-of-the-art wastewater treatment has a significant carbon footprint and this invention is a move towards energy neutral and eventually energy positive treatment facilities. The invention can be used in both sewage and industrial wastewater treatment, and at new plants and as a retrofit addition to existing facilities. In a retrofit scenario, the addition of this invention can also serve to expand the treatment capacity of the existing facility and hence avoiding the need for expansion of existing treatment facilities as needs increase.

Example 1 - F/M ratio effect

Two Sequencing Batch Reactors (SBR) were employed to condition the biomass, which was collected from a wastewater treatment plant. Two different concentrations of synthetic feed were applied to these two reactors, which were 200 mg COD/L and 600 mg COD/L respectively. Therefore, the F/M ratio for the two reactors is 0.15 and 0.45.

From Figure 1, the results suggest that biomass harvested from the low F/M ratio conditions showed better biosorption performance, e.g. 59.7 mg COD/g SS at pH= 8, than the biomass harvested from high F/M condition, e.g. 31.2 mg COD/g SS. The major difference is contributed by the mechanism of bioaccumulation or uptake. Data from Figure 2 concludes that Biomass S1 shows a much higher bioaccumulation capacity than S2 biomass e.g. 35.1

mg COD/g SS vs. 15.2 mg COD/g SS at pH= 7 and 37.3 mg COD/g SS vs. 13.6 mg COD/g SS at pH= 8. The PHA-staining pictures in Figure 3 confirm this hypothesis. There are much more PHA stored in the cells from biomass S1 than the cells from biomass S2. From the lab results, it appears that the optimum pH range for biosorption is from 7 to 8. This was likely because bioaccumulation was inhibited when pH went too low or too high.

After biosorption, the biomass is enriched by the carbonaceous components. The biomass calorific value (CV) therefore also increases respectively. The higher CV can be used as an indication of a potentially large amount of methane production in an anaerobic digestion process. Figure 4 shows the Calorific Value for biomass S1 and S2, both before and after biosorption, under different pH conditions. The largest increase, which is 0.95 kJ/g SS, appears at pH= 8 for biomass S1.

Example 2 - Dissolved Oxygen (DO) effect

This section comprises the investigation of biosorption performance of the biomass conditioned and harvest from two different Dissolved Oxygen (DO) levels. The reactor conditions used for the culture of Biomass D1 was maintained at a low DO level, ranging from 0.5 to 1.0 mg/L. For Biomass D2, the DO level of the reactor for culturing was controlled at a relatively high level, ranging from 5.0 to 6.0 mg/L. Biosorption batch tests were conducted to compare the difference in biosorption performance between biomass D1 and D2.

In the Biomass D2 community, filamentous microbes dominate, as shown in Figure 5. This is mainly due to the high DO applied in reactor D2. In contrast, the Biomass D1 microbial community shows a well aggregated structure.

The biosorption performance for the biomass D1 and D2 under different pH conditions is shown in Figure 6. Biomass D1 had a better biosorption capacity than D2, e.g. 34.3 mg COD/g SS vs. 25.2 mg COD/g SS at pH= 7. The difference was majorly due to the bioaccumulation mechanism. Biomass D2 has very limited bioaccumulation. Although D2 showed a better surface sorption as shown in Figure 7, lack of bioaccumulation still put it at a disadvantage with respect to overall biosorption performance. From the lab investigation, it appears that the bioaccumulation mechanism can contribute a significant portion to biosorption and thus can play an important role in the overall biosorption performance.

CLAIMS

1. A method of treating wastewater, comprising the steps of:

(i) culturing a first and/or a second and/or a third microbe population in a first, a second, and a third vessel, respectively,

the first microbe population being capable of bioaccumulating at least one pollutant,

the second microbe population being capable of producing a high level of Extracellular Polymeric Substances (EPS) and/or being capable of entrapment of at least one pollutant, and

the third microbe population being capable of surface adsorption of at least one pollutant;

(ii) dividing the cultured first and/or second and/or third microbe populations into a first portion and at least a second portion, respectively, wherein each respective at least second portion is capable of regenerating the respective microbe population;

(iii) contacting a main stream of wastewater comprising at least one pollutant with the first portion of the first and/or second and/or third microbe populations, such that the first portion of the first and/or second and/or third microbe population removes at least part of the at least one pollutant from the wastewater; and

(iv) removing at least part of the first portion of the first and/or second and/or third microbe populations from the main stream of wastewater.

2. The method according to Claim 1, wherein steps (i) to (iv) are repeated continuously in a sequential batch or continuous flow process.

3. The method of Claim 1 or Claim 2, wherein, when the second microbe population is present, the first portion of the second microbe population comprises EPS.

4. The method according to any one of the preceding claims, wherein in step (i), the culturing is within a first and/or a second and/or a third vessel that is in fluid contact with a side-stream of wastewater diverted from the main stream of wastewater comprising the at least one pollutant.

5. The method according to any one of the preceding claims, wherein, after step (iv), the removed microbe population(s) is anaerobically digested.

6. The method according to any one of the preceding claims, wherein:

the first microbe population is capable of bioaccumulating at least one pollutant selected from the group consisting of colloidal and/or soluble organic carbon pollutants; and/or

the second microbe population produces EPS capable of removing at least part of the at least one pollutant selected from the group consisting of colloidal and/or particulate organic carbon pollutants; and/or

the second microbe population is capable of removing by entrapment at least one pollutant selected from the group consisting of particulate organic carbon pollutants; and/or

the third microbe population is capable of removing by surface adsorption at least one pollutant selected from the group consisting of colloidal and/or soluble organic carbon pollutants.

7. The method according to any one of the preceding claims, wherein step (iii) comprises:

increasing the presence of the first microbe population in the main stream of wastewater to between about 0.3 g/L to about 3 g/L; and/or

increasing the presence of the second microbe population in the main stream of wastewater to between about 0.3 g/L to about 3 g/L; and/or

increasing the presence of the third microbe population in the main stream of wastewater to between about 0.5 g/L to about 3 g/L .

8. The method according to any one of the preceding claims, wherein step (i) comprises culturing the first microbe population under conditions that promote dominance of microorganisms capable of glycogen accumulation.

9. The method according to claim 8, wherein step (i) comprises culturing the first microbe population using at least one of:

(A) alternating between anaerobic and aerobic phases;

(B) providing feed in the anaerobic phase;

(C) controlling dissolved oxygen levels;

(D) controlling pH levels;

(E) controlling the F:M ratio (feed:mass); and

(F) controlling temperature levels.

10. The method according to claim 9, wherein step (i) comprises at least one of:
    (A) alternating between anaerobic and aerobic phases;
    (B) providing feed in the anaerobic phase;
    (C) maintaining dissolved oxygen levels between about 1.5 mg/L to about 2.5 mg/L during the aerobic phase and/or at about 0 mg/L during the anaerobic phase;
    (D) maintaining pH levels between about 6 to about 8.5;
    (E) maintaining the F:M ratio between about 0.1 to about 0.3; and
    (F) maintaining temperature levels between about 25°C to about 45°C.

11. The method according to any preceding claim, wherein the method comprises the use of a first microbe population being capable of bioaccumulating at least one pollutant.

12. The method according to any preceding claim, wherein the method comprises culturing two or more of a first, a second and a third microbe population for use in subsequent steps.

13. The method according to any preceding claim, wherein the first microbe population, when present, comprises at least one microorganism that is capable of glycogen accumulation.

14. The method according to claim 13, wherein the first microbe population comprises at least one microorganism of the class *Gammaproteobacteria* or *Alphaproteobacteria*.

15. The method according to claim 14, wherein the first microbe population comprises a microorganism of the class *Gammaproteobacteria* that is *Candidatus Competibacter phosphatis* and/or a microorganism of the class *Alphaproteobacteria* that is a *Defluviicoccus vanus*-related organism.

16. The method according to any preceding claim, wherein the first microbe population comprises at least one microorganism with:

    a biosorption capacity of at least about 40 mg COD/ g SS (Chemical Oxygen Demand per gram of Suspended Solids); and/or

a bioaccumulation capacity of at least about 20 mg COD/ g SS (Chemical Oxygen Demand per gram of Suspended Solids); and/or

a biomass calorific value increase of at least about 0.5 kJ/ g SS (kilojoules per gram of Suspended Solids), between step (i) and step(iv).

17. The method according to any preceding claim, wherein the time of contacting in step (iii) is at least about 10 minutes.

18. The method according to any preceding claim, wherein the second microbe population comprises at least one microorganism isolated from a culture of microbes with a high level of EPS.

19. The method according to any preceding claim, wherein the high level of EPS is at least about 150mgEPS/gVSS.

20. The method according to any preceding claim, wherein the second microbe population comprises at least one microorganism isolated from a culture of microbes with cellular morphology suitable for entrapment of at least one pollutant by enmeshment.

21. The method according to any one of claims 18 to 20, wherein the at least one isolated microorganism is optionally identified, and is further cultured in nutrient enriched media to obtain the second microbe population, wherein the nutrient enriched media is optionally optimized to encourage growth of the optionally identified isolated microorganism.

22. The method according to claim 20 or 21, wherein the at least one isolated microorganism is further cultured with an F:M ratio of between about 0.1 to about 0.3.

23. The method according to any preceding claim, wherein the second microbe population, when present, comprises at least one species selected from the group consisting of *Pseudomonas sp., Bacillus sp., Pantoea sp., Serratia sp., Yersinia sp., Microbacterium sp., Enterobacter sp., Photorhabdus sp.*.

24. The method according to any preceding claim, wherein step (i) comprises culturing the third microbe population using at least one of:

(A) low F/M;

(B) nutrient deficiency;

(C) low dissolved oxygen levels;

(D) readily-metabolizeable substrates;

(E) high Sludge Retention Time (SRT); and

(F) presence of hydrogen sulfide.

25. The method according to claim 24, wherein step (i) comprises maintaining, in the third vessel, at least one of:

the F:M ratio at between about 0.1 to about 0.3;

a nitrogen concentration of less than 10mg/L;

a phosphate concentration of less than 1 mg/L;

a dissolved oxygen level of between about 0.1 mg/L to about 1.5 mg/L;

a level of readily-metabolizeable substrates of about 150 mg/L, wherein the readily-metabolizeable substrates are selected from the group consisting of low molecular weight organic acids and simple sugars;

a Sludge Retention Time (SRT) of at least about 50 days; and

the presence of hydrogen sulphide.

26. The method according to any preceding claim, wherein the third microbe population, when present, comprises at least one species selected from the group consisting of *Thiothrix sp., filamentous bacteria Type 0914, filamentous bacteria Type 0411 (Flexibacter subgroup of Flexibacter-Cytophaga-Bacteriodetes phylum), and 0961, Nocardioforms, Nostocoida Limicola II and III.*

27. The method according to any preceding claim, wherein step (ii) further comprises treating the respective first portions of one or more of the first, second and/or third microbe populations.

28. The method according to claim 27, wherein the second microbe population is present and the treated first portion of the second microbe population has reduced or no further growth after contacting the main stream of wastewater.

29. The method according to any preceding claim, wherein the first microbe population is used.

30. The method according to claim 29, wherein:

step (i) comprises culturing the first microbe population in the first vessel, the first microbe population being capable of bioaccumulating at least one pollutant;

step (ii) comprises dividing the cultured first microbe population into a first portion and at least a second portion, wherein the at least second portion of the first microbe population is capable of regenerating the first microbe population;

step (iii) comprises contacting the main stream of wastewater comprising at least one pollutant with the first portion of the first microbe population, such that the first portion of the first microbe population removes at least part of the at least one pollutant from the wastewater; and

step (iv) comprises removing at least part of the first portion of the first microbe population from the main stream of wastewater.

31. An apparatus for wastewater treatment, comprising one or more culturing vessels for culturing microbe populations, said culturing vessels being in fluid communication with at least one contact vessel for contacting portions of cultured microbe populations with a main stream of wastewater, wherein the at least one contact vessel is in fluid communication with the main stream of wastewater.

32. The apparatus of claim 31, wherein at least one of the one or more culturing vessels is in fluid communication with a side-stream of wastewater diverted from the main stream of wastewater.

33. The apparatus according to claim 31 or 32, wherein at least one of the one or more culturing vessels is adapted to:

(A) controlling dissolved oxygen levels; and/or

(B) providing feed; and/or

(C) controlling the F:M ratio (feed:mass); and/or

(D) controlling pH levels; and/or

(E) controlling Sludge Retention Time (SRT); and/or

(F) controlling temperature levels; and/or

(G) controlling hydrogen sulphide levels.

34. The apparatus according to any one of claims 31 to 33, wherein the apparatus is adapted to run a sequential batch or continuous flow process to treat the wastewater.

35. The apparatus according to any one of claims 31 to 34, wherein the at least one contact vessel is arranged upstream of an aerobic treatment vessel adapted to receive the wastewater.

36. The apparatus according to any one of claims 31 to 35, wherein the at least one contact vessel is arranged upstream of an anaerobic treatment vessel adapted to receive at least one portion of cultured microbe population that has been contacted with the main stream of wastewater.

37. The apparatus according to any one of claims 31 to 36, wherein the at least one contact vessel is configurable as a primary settling tank for liquid-solid separation of the main stream of wastewater.