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Review

Methanotrophy: From the environment to industry and back

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ABSTRACT

Understanding concerning natural microbially mediated processes has been severely retarded by the common requirement to study microorganisms only as pure monocultures under aseptic conditions, in spite of the fact that, in virtually all real environments, microbial strains function in concert. This is particularly true in processes involving multiple elemental cycles and system segregation, as is the case for methanotrophic bacterial consortia functioning in nature. Although such consortia have exhibited a clear and important role in both natural and engineered aquatic systems, methanotrophic bacteria were largely excluded from detailed physiological study until the prospects for microbially derived animal feed and human food protein or single cell protein (SCP) production became a commercial objective in the 1960s and 1970s. This resulted in a wealth of both physiological and bioprocess information concerning methanotrophic consortia, which was, until recently, largely ignored as far as natural environmental processes were concerned. However, as concern about greenhouse gas, particularly methane and nitrous oxide emissions, from non-point aquatic sources increased, previously accumulated information from the development of commercial SCP production has become invaluable both in understanding factors affecting emission patterns and in the development of technology for emissions control. In the present commentary, the potential of mobile unconstrained membrane methanotrophic biofilm reactors for dissolved methane oxidation and possible dissolved nitrous oxide reduction to dinitrogen is introduced.

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1. Introduction

The present discussion concerns reactions mediated by methanotrophic bacterial consortia in perturbed natural aquatic environments and in engineered bioprocesses. In recent years, several important papers that impact on realistic investigations concerning the role of microorganisms in such environments and processes have appeared. These are:

Microbial diversity: the importance of exploration and conservation [1];
Prokaryotes: the unseen majority [2];
Biofilm: city of microbes [3]; and
Synergistic interactions in the microbial world [4].

Essentially, these publications emphasized the scope, the magnitude and scale, and the complexity of microbial activity in real, unprotected situations, unlike the historical approach to microbiology that had been dominated by the monoculture philosophy that stemmed from the work of Koch in Berlin in the early 1880s on the development of effective procedures for the isolation of pure cultures of bacteria. Although Koch's success revolutionized the entire experimental tradition of microbiology, it was also responsible for more than 100 years, for the widespread failure amongst microbiologists to recognize that it is intra- and inter-consortia interactions that represented the real situation pertaining to microbial activity in unprotected environments. Unprotected environments include both perturbed and unperturbed natural eco-systems and engineered environments, including the vast majority of waste biotreatment processes and a small number of industrial bioprocesses. In addition, a hierarchy concerning the relative importance of the various sectors of microbiological research has been established. Essentially, studies at the molecular level are considered to be the most important, followed by studies concerning individual cells, studies concerning pure cultures (populations) and, finally, studies of defined mixed cultures (consortia), in descending order of perceived importance. This has resulted in a major imbalance in the availability of appropriate microbiological data and, hence, our ability to develop appropriate hypotheses concerning the overall functioning of microbial systems, particular systems involving microbial consortia, and their involvement in a broad spectrum of microbially mediated geochemical processes. In the context of microbial consortia, several definitions exist: single primary substrate utilizing strains supported by several ancillary strains that do not themselves utilize the primary substrate, but contribute in various ways to the efficiency of primary substrate

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utilization [5]; mixed cultures exhibiting cooperative metabolism in which each component strain is responsible for a step or steps in the biodegradation of a particular compound [6]; bacterial communities in which the horizontal transfer of genes and their incorporation into recipient strains occurs [7]. Essentially, a consortium achieves the same role as does a genetically manipulated (or engineered) strain, but with superior stability, because genetically engineered strains are, invariably, both physiologically unbalanced and fastidious.

The major thesis of this communication is that much of the research undertaken in support of industrial SCP production from the methane fraction of natural gas using methanotrophic bacterial consortia has direct application in the control of greenhouse gas emissions from perturbed natural and engineered aquatic eco-systems. In the development of SCP production processes, microbiologically mediated processes were, virtually for the first time, subjected to both realistic process engineering evaluation and appropriate microbiological research, with a view to achieving optimum culture performance.

In processes mediated by aerobic methanotrophic and other aerobic gaseous substrate-utilizing bacteria, the key process efficiency criteria are substrate and oxygen conversion coefficients, rather than substrate and oxygen based yield coefficients. In general, it can be said that in the case of commercial aerobic gaseous carbon energy substrate based processes, both carbon energy substrate and oxygen conversions must exceed 80% to ensure economic viability, while in the case of engineered environmental aerobic methane elimination processes, oxygen conversion must approach 100% and methane conversion must exceed 80% to ensure acceptable economic and pollutant elimination performance. The types of bioreactor that have found application in commercial aerobic methane based processes employ extremely high power inputs in order to achieve the very high methane and oxygen gas–liquid transfer rates commensurate with both high gas phase substrate and nutrient conversions and very high bioreactor productivities. However, in the case of environmental aerobic methane elimination processes, acceptable process performance must be achieved with minimum power input. Accordingly entirely different bioreactor systems must be considered for such processes. These include biofilters, bio-trickling filters (bioscrubbers) and membrane aerated biofilm reactors (MABRs), the last of which can, under particular operating conditions, be configured so as to achieve either very high gaseous substrate or nutrient conversion. The present contribution examines the possibilities that mobile shell-free MABRs might offer as an effective solution for *in situ* dissolved methane and nitrous oxide reduction in large fresh and marine water bodies from which methane and nitrous oxide emissions to atmosphere occur.

2. Atmospheric pollution issues

Historically air pollution control was primarily concerned with smoke (ultra-fine particulate matter), aerosol (mist) and odour elimination. Less immediately obvious air pollutants, such as invisible, odourless gases were disregarded until more recent times when sensitive analytical techniques for a wide spectrum of gaseous phase pollutants became available. Two issues, stratospheric ozone depletion and greenhouse gas emissions contributing to significant global warming have, in recent decades, dominated consideration of atmospheric pollution, and have, by way of political response, resulted in the Montreal Protocol, as far as specific anthropogenic compound (pollutant) release on the ozone layer, and the Kyoto Protocol, as far as greenhouse gases responsible for both direct and indirect radiative forcing (global warming) are concerned. The former protocol has proved generally acceptable and effective as far as specific emissions are concerned, but the latter remains embroiled in political controversy both with respect to either compliance or non-compliance, on the one hand, and possible national emissions control based on either rigid command and control regulations (reduction or carbon capture) or pragmatic, market-based, cap-and-trade approaches, particularly as far as carbon dioxide is concerned, on the other hand. In the latter context, this has allowed the concept of carbon emissions trading between over-producing industries, such as power generation from fossil fuels, oil refining and cement manufacture, in developed countries and non-industrialized developing countries that offer extensive natural or constructed photosynthetic carbon dioxide sink capacity, to be established.

Human-induced climate change has become synonymous with increasing atmospheric carbon dioxide concentration, but there are many other atmospheric components that contribute to climate change [8]. After water vapour and carbon dioxide, methane and nitrous oxide are the most important long-lived atmospheric components (greenhouse gases) responsible for radiative forcing [9] and it has been suggested that carbon dioxide, methane and nitrous oxide are the 'managing' greenhouse gases, while water vapour is the chief responding gas [10]. Although both methane and nitrous oxide exhibit mass warming potentials of one and two orders of magnitude greater than carbon dioxide, respectively, their atmospheric concentrations are markedly lower. Most systems proposed for climate change amelioration seek to reduce the mass of carbon dioxide emissions from anthropogenic sources. However, such policies fail to recognize probable physiological benefits such as enhanced crop yields that can result from increased atmospheric carbon dioxide concentrations, whereas methane is physiologically inert as far as plants are concerned [8]. Hence, in more holistic approaches concerning the impact of greenhouse gases, radiative forcing should not be considered in isolation. As a result, Cox et al. [8] have suggested that anthropogenic and natural methane emission control might provide an important alternative approach for future climate change amelioration. Methane is, of course, the primary product of natural anaerobic digestion (complete mineralization of carbonaceous matter) by methanogenic microorganisms and natural gas hydrate decomposition, while nitrous oxide is an intermediate product of incidental, sequential nitrification/denitrification, generally resulting from excessive application of nitrogenous (ammonium and urea based) fertilizers to agricultural land, which ultimately occurs in surface water bodies after run-of.

Waste gas biotreatment has developed as a novel end-of-pipe technology for the removal of a significant fraction of specific gaseous and vapour phase pollutants from point source waste gas, most frequently waste air, streams. However, neither natural nor constructed surface water bodies produce point sources with the possible exception of the turbine channels of power generating hydroelectric dams [11]. In general, the release of gaseous emissions is distributed over the entire surface area of any particular water body. Hence, conventional waste gas biotreatment, in the form of either biofilters or bio-trickling filters, is neither appropriate nor effective. What is needed is a system that can provide *in situ* biotreatment of methane and/or nitrous oxide, in the dissolved state, prior to release by ebolution at the water surface. One such system that would offer real potential in this respect would be a submerged form of membrane aerated biofilm reactor (MABR), a type of bioreactor that has considerable potential for wastewater biotreatment [12].

3. Methanotrophy and the carbon cycle

Methane, or marsh gas, was discovered by Volta in 1776, as bubbles from the marshy surround of Lago Maggiore. Methane bubbles that were emitted from the anaerobic lake sediment were considered to result from the recycling of dead biological matter. Essentially, what Volta observed is known today as methanogenesis, a strictly anaerobic process. A little more than 100 years ago, Sohngen [13] and Kaserer [14], essentially simultaneously but independently, described the isolation, or more probably the enrichment, of a methane-oxidizing bacterium, *Bacillus methanicus*, from aquatic sources. Later, Sohngen [15] stressed that in nature, *Bacillus methanicus* was found in association with several species of fresh-water plants. Shortly after the discovery of bacterial methane oxidation, Jensen [16] included *Bacillus methanicus*, renamed *Methanomonas methanica*, in a scheme of microorganisms active in natural eco-systems. While it might have been expected that this would have stimulated widespread interest in bacterial methane oxidation as a natural phenomenon counteracting methane emissions from anaerobic sediments, few publications concerning methane-oxidizing bacteria appeared in the next 40 years, most probably because conventional microbiological laboratory techniques were unsuited to studies involving potentially explosive substrates. However, one study by Harrison and Subramania-Aiyer [17] was of ecological significance. They investigated the impact of the gases of swamp rice soils on the aeration of the roots of the rice crop and hypothesized that the soil immediately in contact with the irrigation water was covered with an organized microbial film (biofilm) which, on the one hand, emits oxygen rich gas, while on the other hand, arrests and assimilates methane. The most probable mechanisms occurring in the biofilm were methane oxidation by methane-utilizing bacteria and concomitant photosynthetic oxygen production from carbon dioxide by green algae functioning synergistically within the biofilm.

A broad understanding of the growth physiology of methane-oxidizing monocultures was finally developed in work by Dworkin and Foster [18], Leadbetter and Foster [19] and Foster and Davis [20], while the superior growth characteristics of mixed methane-utilizing enrichment cultures were demonstrated by Hamer et al. [21], Vary and Johnson [22] and Sheehan and Johnson [23]. The first of these enrichment cultures was subsequently shown to be a methane-utilizing consortium by Wilkinson et al. [5] and formed the basis for using such consortia in methane (natural gas) based single cell protein (SCP) manufacturing processes. It should also be noted that in the case of the nitrogen cycle, methane-utilizing consortia show markedly broader activity and versatility than do methane-utilizing monocultures.

4. Single cell protein from methane

Inadequate supplies of high quality protein for human nutrition were identified as a major cause of malnutrition in the 1950s. This stimulated a search for alternative protein sources for incorporation in both human food and compounded animal feeds and resulted in industrial production routes for microbial protein (SCP) from feedstocks which were entirely inaccessible to mammalian nutrition. Amongst possible feedstocks, particular attention was directed towards waxy *n*-alkanes, a troublesome impurity in gas oil, as a possible refining step, purified waxy *n*-alkanes, the methane fraction of natural gas and methanol, produced by chemical synthesis from natural gas. Although the three latter feedstocks all resulted in commercial-scale SCP production, SCP failed to make a significant quantitative contribution to Global feed and food protein supplies, which are, of course, dominated by soybean protein production, primarily in the USA, but with major contributions from both Brazil and Argentina in recent years. The apparent commercial attractiveness of SCP resulted from the extremely low crude oil and natural gas prices that had existed prior to the first OPEC mediated step increase in crude oil prices in 1973. Even at pre-1973 crude oil and natural gas prices, SCP was, contrary to popular belief at that time, never destined to alleviate the plight of the chronically malnourished. However, it should be noted that in the past decade, the direct natural gas (methane) route for SCP production has been successfully used for protein manufacture for the high value farmed salmon market [24]. As far as functionality is concerned SCP is employed as an essential nitrogen component in food and feeds.

5. Methanotrophs and the nitrogen cycle

The versatility of methane-oxidizing cultures and consortia with respect to their nitrogen metabolism far exceeds that of most individual microbial strains. Circumstantial evidence that natural gas-utilizing bacteria fix dinitrogen existed since the 1930s, but methanotrophic dinitrogen fixation was only finally confirmed by Davis et al. [25] and generally accepted after de Bont and Mulder [26] demonstrated the inadequacies of the acetylene reduction test for assessing dinitrogen fixation by various methanotrophs. Ammonium oxidation by obligate methanotrophic bacteria was first reported by Hutton and ZoBell [27] and was subsequently investigated in detail by Dalton [28] and by O'Neill and Wilkinson [29]. However, these studies indicated that oxidation only proceeded as far as nitrite. A short time later Drozd et al. [30] demonstrated that various types of methanotrophs were able to nitrify ammonia via nitrite to nitrate, *i.e.*, mediate complete nitrification, suggesting that methanotrophic consortia might compete equally with chemo-autotrophic nitrifying consortia in aquatic environments where both methane and oxygen are available.

Although methane has repeatedly been suggested as an appropriate carbon substrate for denitrification [31–34], to date, actual denitrification by methanotrophic monocultures has never been validated [35]. Most probably, in some of the studies in which such claims were made, the methanotrophic culture employed was a consortium which included a denitrifying methanol utilizing strain as a key anicillary bacterium growing on by-product methanol.

In many considerations of the biogeochemical nitrogen cycle, dinitrogen is the only gaseous product deemed worthy of mention, even though nitrous oxide was identified as a product of denitrification by Beijerinck and Minkman [36], it was subsequently omitted from most schemes for denitrification until relatively recent times, *e.g.*, Robertson and Kuenen [37] and Kuenen and Robertson [38], when the temperature forcing effects of nitrous oxide in the atmosphere was becoming evident. This was in spite of the fact that Wijler and Delwiche [39] emphasized nitrous oxide as a product of denitrification in soil, while Yoshida and Alexander [40] reported nitrous oxide release from ammonia oxidation by both chemo-autotrophic and chemo-heterotrophic bacteria. Additionally, Yoshinari [41] demonstrated nitrous oxide formation from ammonium compounds by a methanotrophic bacterium, but as growth was not supported, attributed this to cometabolic activity. It should also be noted that Jones and Morita [42] reported widespread methane oxidation capacity in both chemo-autotrophic ammonium-oxidizing and nitrite-oxidizing bacteria, an observation that was confirmed in the case of the former bacteria by Ward [43,44].

The reintroduction of nitrous oxide into the nitrogen cycle clearly indicated its formation during both nitrification and denitrification processes and together with the fact that combined nitrification–denitrification processes can take place simultaneously in some microbial communities that mediate nitrogenous pollutant removal from wastewater [45]. These researchers stressed the importance of rapid and accurate gas phase analyses in providing full data for such processes, a feature widely omitted from the vast majority of studies until recently. Quantitative studies concerning bacterial nitrous oxide production remained, as far as either methanotrophs or methanotrophic consortia are concerned, nonexistent.

Waki et al. [46,47] examined the effects of changes from aerobic to anaerobic conditions and *vice versa* in both batch and continuous cultures of *Paracoccus denitrificans*, but without either gas phase analyses or use of a growth medium free from complex organic nitrogen compounds, thus making the results obtained difficult to interpret. However, a decade later, Omlin [48] revisited the effects of oxic-anoxic step changes in pH controlled continuous cultures of the same bacterium in a defined mineral salts growth medium with pyruvate as the carbon energy substrate and where either nitrate or nitrate–ammonium mixtures were used as nitrogen sources. Prior to a step change from steady state oxic to anoxic conditions at any pre-selected pH or nitrogen source availability, neither nitrous oxide nor dinitrogen production were observed, but under transient state operating conditions, both significant nitrous oxide and dinitrogen production occurred. A step change with nitrate as nitrogen source at pH 6.75 resulted in markedly increased nitrate consumption with initial short term nitrite accumulation, low levels of dinitrogen production and significant nitrous oxide production, which, on a molar basis, exceeded dinitrogen production by some 10 times, even after anoxic steady state conditions had been established. When step changes were conducted at pH 6.75 and at pH 7.5 with nitrate and ammonium as nitrogen sources, ammonium consumption was unaffected, while nitrate consumption increased markedly. An initial peak in dinitrogen production occurred. This was followed by a significant peak in nitrous oxide production were, but upon establishment of an anoxic steady state, nitrous oxide production became essentially zero. Anoxic steady state cultures growing with nitrate alone produced significant quantities of nitrous oxide relative to dinitrogen, whereas anoxic steady state cultures growing with nitrate and ammonium exhibited complete denitrification with only dinitrogen produced, a probable result of a shortage of reducing equivalents for respiration in cases where nitrate had to be reduced to ammonium prior to assimilation. Also of importance is the fact that after step changes from oxic to anoxic conditions, *Paracoccus denitrificans* commenced denitrification immediately, indicating that even during growth under oxic conditions this bacterium had the complete set of enzymes necessary for denitrification. Clearly, transient state operating conditions markedly affect nitrous oxide formation.

The above discussion concerns *Paracoccus denitrificans* and no evidence exists that this bacterium is a component of methanotrophic consortia. However, Lloyd et al. [49] suggested similar behaviour in the case of other denitrifying bacteria. The first denitrifying strain that was shown to be a component of methanotrophic consortia was a methylotrophic *Hyphomicrobium* sp. [50]. This strain, when growing exponentially under oxic batch culture conditions with ammonium nitrate as the supplied nitrogen source, showed a remarkable deviation with respect to the oxygen based biomass yield coefficient when the dissolved oxygen concentration in the growth medium fell below 25% of saturation with air, a deviation that could only be explained by nitrate, rather than oxygen, becoming the preferred electron acceptor. Essentially, the affinity of the *Hyphomicrobium* sp. for nitrate exceeded that for oxygen, a feature that enhanced its competitive ability, relative to methanotrophs, in methanotrophic consortia at reduced dissolved oxygen concentrations. The role of both individual methanotrophs and methanotrophic consortia in the biogeochemical nitrogen cycle has, very largely been under estimated, even ignored. Such matters require immediate correction.

6. Renewable energy and changing patterns of land use

Currently, many parts of the World are placing major emphasis on renewable sources of energy, particularly the production of biofuels from sugar cane, cereals and oil seeds, thereby initiating competition for such commodities between their traditional uses as food and feed and newfound uses for biofuel production. This will tend to reverse a trend towards the replacement of rice by imported wheat, thereby increasing intensive wet land cultivation of rice in Asia, although innovative management of water regimes, soil and nitrogen availability offer prospects for regulating both nitrous oxide and methane emissions from rice fields [51]. Crop demand for biofuel production will also increase incentives to convert tracts of tropical rain forest into crop producing land, involving massive surface soil disruption with associated greenhouse gas emissions and subsequent synthetic nitrogenous fertilizer application. A further effect from the drive towards increased renewable energy production is actual and proposed increased investment in hydroelectric power generation, which frequently involves flooding forest and other terrestrial vegetation. Examples of this exist in both tropical [52] and sub-arctic peat land [53,54] regions where, in both cases greenhouse gas emissions have proved to be considerable, rather than nonexistent as originally portrayed by the hydroelectric power generation industry. In fact, it has even been proposed that methane collection for fuel be considered in Brazil, but gas ebullition in tropical reservoirs tends to be reservoir wide [55], thereby introducing serious collection difficulties. However, if major methane release can be restricted to the turbines and associated race-ways [11], where cavitation will undoubtedly enhance dissolved gas stripping, such collection might prove feasible.

In a completely different context, constructed wet lands are increasingly being recommended for sewage and wastewater treatment and constructed coastal mangrove plantations are being funded from carbon dioxide emissions charges, without appropriate environmental impact assessments, particularly with respect to their own emissions of temperature forcing gases, primarily methane and nitrous oxide. What is clear is that such initiatives will, in spite of apparent environmental advantages, result in greatly increased methane and nitrous oxide emissions in the case of the former [56,57] unless appropriate remedial actions are taken, while artificially constructed mangrove plantations will result not only in gaseous emissions, but also the possible release of organic vapours from foliage. However, such emissions will probably remain largely insignificant compared with the long-term effects resulting from continuing natural wetland destruction that is continuing, unabated, on a global scale.

7. Climate control systems

In recent years directives have been applied by the governments of some industrialized, developed countries to markedly reduce greenhouse gas, particularly carbon dioxide, emissions. Overall evaluation of the cost of energy generation and liquid fuel production from fossil feedstocks (resources) suggests a worldwide capital expenditure measured in trillions of dollars. At the present time, the cumulative capital cost of sustainable energy generation schemes based on renewable and recurring resources is measured in billions of dollars. Parallel with renewable energy initiatives, non-photosynthetic carbon capture technologies based on either underground or marine immobilization and storage, are being proposed, with probable capital investment costs measured in billions of dollars. The deliberate large-scale manipulation of the planetary environment, frequently referred to as geoengineering, to counteract anthropogenic climate change has recently been discussed in detail [58]. Two distinct approaches exist; carbon dioxide removal

(CDR) technologies and solar radiation management (SRM) technologies. The latter will not be discussed further here. The object of CDR technologies is to remove carbon dioxide from the atmosphere by biological, physical or chemical means operating either on land or in the oceans. Terrestrial biological approaches include afforestation, reforestation and avoidance of deforestation, thereby establishing terrestrial carbon sinks in which carbon dioxide can be sequestered in soil and in growing plant biomass, as well as balanced bioenergy (biofuel)/carbon dioxide sequestration schemes and sequestration by burial of either forestry or crop wastes or biochar (charcoal). Proposed oceanic CDR technologies include iron fertilization, phosphorus/nitrogen fertilization and enhanced upwelling/downwelling. Chemical approaches on both land and in the ocean seek to achieve enhanced weathering. On land both *in situ* and techniques involving the dispersion of basic minerals, *e.g.*, olivine, in agricultural soils have been proposed. In the oceans, proposed technologies involve the dispersion and dissolution of previously mined/quarried and size reduced limestone, silicates or calcium hydroxide to achieve alkalinity enhancement. Land based physical methods involve atmospheric carbon dioxide scrubbers, while proposals for the oceans changes to overturning circulation.

Should CDR approaches and technologies either fail or prove only partially effective, SRM systems seem likely to receive some favourable governmental reaction in spite of no comprehensive environmental risk assessment and, in some cases, estimated capital investment measured in billions of dollars. This suggests that *in situ* biotreatment technologies for greenhouse gas emissions control, specifically methane oxidation and nitrous oxide reduction systems, costing millions of dollars in capital investment, might also become politically acceptable solutions for markedly reducing greenhouse gas emissions from a broad range of engineered, perturbed and pseudo-natural aquatic environments.

In all shallow and moderately shallow surface water bodies, the tendency exists for the natural micro-biota to accumulate as biofilms both at solid-liquid and at liquid–gas interfaces. The concentration of micro-biota comprising such biofilms exceeds, by orders of magnitude, concentrations in the water column and provided drastic limitation with respect to the availability of essential substrates and/or nutrients does not occur throughout such biofilms, significant biodegradative capacity occurs. However, for the most part, liquid-gas interfacial biofilms are relatively unordered and, depending on surface flow regimes, physically unstable. Hence, a bioreactor in which such difficulties with active biofilms does not occur, such as unconstrained (shell-free) MABR modules, would seem to offer very considerable scope as far as both methane bio-oxidation and nitrous oxide bio-reduction are concerned.

8. Biotreatment of gaseous emissions

Microbiological waste gas treatment represents a major development in environmental biotechnology. Until some 50 years ago the concept of using microorganisms for pollutant removal from gas streams had not been given serious consideration [59]. However, more effective enforcement of increasingly stringent environmental legislation, particularly in Europe, has forced hitherto largely neglected possibilities for effective treatment to be applied to various emissions [60]. The desire to exploit the potential of waste gas biotreatment has resulted in the development of apparently diverse biotreater designs; specifically biofilters and bio-trickling filters. Such systems have dominated the biotreatment of waste gas streams from point sources.

The first reported example of the use of methane-utilizing cultures for the elimination of methane from atmospheres was that by Yurovskii et al. [61] concerning coal mines, where it was proposed that active cultures should be coated on the internal surfaces of mines in order to prevent potentially explosive coal mine atmospheres. Whether such explosion prevention measures were effective under practical operating conditions remains unclear, but widespread use of the technique was never reported. Subsequently, further studies concerning underground methane explosion prevention were conducted by Apel et al. [62] and by Sly et al. [63]. The former concerned the application of a biofilm reactor operating with extremely long gas phase residence times which achieved entirely acceptable levels of methane elimination. The latter study concerned a continuous flow bio-trickling filter operating under very low inlet gas phase methane concentrations and moderately long gas phase residence times of 15–20 min, although acceptable levels of methane elimination were achieved. Such relatively low rates of methane elimination undoubtedly result from the relatively low water solubility of methane. More recent studies concerning methane elimination using biofilters have been conducted by Nikiema et al. [64] and by Park et al. [65] and using stirred tank bioscrubbers and bio-trickling filters by Rocha-Rios et al. [66]. In the first of these, only relatively low rates of methane elimination were reported, particularly when compared with the rates of methane utilization that were achieved in process research aimed at SCP production, but Park et al. [65] demonstrated essentially complete methane elimination at 5% methane inlet concentration, but only *ca.* 10% elimination at 25% methane inlet concentration, a difference that was attributed to probable oxygen limitation. In the research reported by Rocha-Rios et al. [66], silicone oil was added to the aqueous phase in both the bioscrubber and the bio-trickling filter in order to enhance methane absorption, but although improved methane elimination performance was achieved, rates remained far lower than can be achieved in very high power input bioreactors which, needless to say, are inappropriate for virtually all environmental applications. Further, this work confirmed the dependence of effective methane elimination on its water solubility.

However, in the case of distributed gaseous emissions from aquatic systems of the type under consideration here, an entirely different approach is needed. Although ebullition of methane has been observed in hydroelectric turbines, the major portion of methane and nitrous oxide release into surface waters are, initially, in the dissolved state. Hence, the problems of treatment that must be resolved concern dissolved methane and nitrous oxide biodegradation, prior to their possible release to atmosphere. Some years ago, Reij et al. [67] proposed the use of membrane bioreactors for waste gas biotreatment, but the several designs proposed, unlike unconstrained membrane MABRs, were only suitable for point source emissions.

9. Unconstrained membrane biofilm reactors?

MABRs were proposed in the 1990s to circumvent the need for either air or oxygen bubbles that might strip either dissolved gases or miscible volatile liquid compounds from wastewater undergoing aerobic biotreatment [68]. Their construction had become possible because of the availability of a range of tubular semi-permeable reinforced membranes and membrane modules that had been originally developed for cross-flow micro-filtration and other membrane separation technologies. Most MABRs are of the shell and tube configuration, comprising a pressurized air or oxygen containing membrane lumen operating with either very low gas throughput rates or in a dead-end mode. Oxygen diffuses through the membrane into a stratified biofilm, submerged in polluted wastewater that flows, through the outer shell, over a membrane attached biofilm, in such a manner that specific dissolved carbonaceous and nitrogenous pollutants initially present in the wastewater are eliminated by either bio-oxidation in the oxic layers or bio-reduction in the anoxic/anaerobic layers of the biofilm. A particular advantage of MABRs is their ability to achieve close to 100% oxygen conversion when operating with 95% pure oxygen, produced by swing absorption, in the dead-end mode. The primary difference with the system proposed here, the unconstrained membrane aerated biofilm reactor, is that the membrane module, without shell, but attached to buoyancy tanks, is located directly in a water body containing dissolved methane and/or nitrous oxide, such that it can move throughout the water body on networks of steel ropes. However, it too can be expected to achieve very high oxygen conversions, when operating with near pure oxygen in the membrane lumen, which is a major advantage in such engineered environmental process systems.

Mixed culture microbial biofilms represent the ultimate in microbial consortia and, as such, are of key importance in the mediation of reactions in both natural aquatic and engineered environmental processes. In fact, as far as the former are concerned, neither discretely dispersed microorganisms nor macro-organisms play a comparable role as far as the cycling of elements are concerned. Until about 1990, the understanding of biofilm function was largely based on unconfirmed hypotheses, but since then, many important advances have been made with respect to understanding biofilm structure, function and physiology. It has even been suggested that analogies might exist between mature biofilms and the tissues of higher organisms [69], while Costerton et al. [70] have also demonstrated that biofilms are organized communities with apparent functional heterogeneity. Biofilms can comprise either pure monocultures or mixed cultures (microbial consortia). Pure monoculture biofilms generally exhibit active effective lifetimes measured in days or weeks, but in contrast, naturally immobilized consortia approach immortality, possibly because of the differentiation of individual strains into self-sustaining clusters.

The mixed culture biofilms that develop in engineered environments can be considered to be semi-natural biofilms. The limiting substrates/nutrients for biofilm activity are the carbon substrate and oxygen which are, in the case of MABRs, supplied from different sides of the biofilm. In conventional biofilms attached to non-permeable solid support materials, the active oxic layer is, typically, 50–200 μm in thickness, which corresponds directly to the depth of oxygen penetration into the biofilm. The bacteria involved in such biofilms are obligate aerobes and both facultative and obligate anaerobes, while the distances dividing fully aerobic, anoxic and strictly anaerobic layers is frequently only 1–2 μm. Of course, the sequence between such layers in conventional biofilms varies for those observed in membrane grown biofilms. In all layers of mature biofilms endogenous activity, death by lysis and subsequent cryptic growth occur, while overall growth is minimal, with the vast majority of the component bacteria being in a non-dividing (stationary) state where significant growth, but not significant biochemical activity, has ceased.

The potential role played by methanotrophs in the biosphere was first highlighted by Higgins et al. [71] and the same reactions, involving both cometabolism and fortuitous oxidation of potentially noxious pollutants can, obviously, also be harnessed in engineered biotreatment processes. Most initial attempts to achieve this involved multi-stage submerged culture bioreactor systems employing discretely dispersed mixed methanotrophic cultures [72], in spite of the fact that Wilkinson and Hamer [73] had already demonstrated the ability of methanotrophic consortia to grow as biofilms. Finally, it was Clapp et al. [74] who realized the advantages that might accrue from the use of MABRs for the aerobic cometabolic oxidation of trichloroethylene using methane as the primary carbon energy substrate. Both oxygen and methane were supplied by diffusion from the membrane lumen of the MABR, while the trichloroethylene was present, dissolved in water containing necessary mineral salts on the shell side of the MABR. The safety, with respect to possible explosion, of such a configuration was subsequently questioned and a MABR system in which oxygen was supplied from the membrane lumen and methane was dissolved in the polluted wastewater, circulated to the shell side of the system, was proposed by Rishell et al. [75] for such biotreatment processes. This provided a counter diffusional situation in the mixed methanogenic biofilm and it is obvious that such a system could also be used for effective dissolved methane elimination by shell-free MABRs. In such systems, dissolved methane transfer rates into the biofilm from the water body will be enhanced as the membrane module, mounted and guided by retractable submerged steel ropes, traverses the water body that is subject to treatment.

Nitrous oxide reduction has been ignored in the previous paragraph. The reason for this is that the performance of methanotrophic consortia in mediating nitrification–denitrification processes requires further study in order to accumulate a much larger body of knowledge, particularly concerning the mechanisms of reactions involving nitrous oxide reduction to dinitrogen and the factors that impact on such reactions. Suffice to say, any such developments will, most probably, involve intentionally stratified microbial biofilms that have been developed prior to use in separate biofilm deposition systems.

10. Concluding remarks

Environmental biotechnology cannot be exclusively based, as are other sectors of biotechnology, on genomics and proteomics. While this does not preclude consideration of metabolic fluxes, it is a systems biological approach that is needed to develop both greater understanding and possibilities for control. It must be hoped that the employment of molecular probes for the identification of, for example, multiple methanotrophic strains in specific methanotrophic environments rather than non-methanotrophic components present in such consortia, will not become an activity within itself and that future studies concerning pseudo-natural, perturbed and engineered aquatic environments in which methanotrophic consortia both thrive and mediate key biogeochemical processes that either reduce or prevent greenhouse gas emissions from such environments will emphasize performance criteria rather than taxonomy. The proposals presented in this contribution remain, at least in part, hypothetical. However, they do indicate the scope of ideas and concepts that process oriented environmental biotechnology, essentially a branch of systems biology, can offer in the probable solution of major environmental problems. Bioprocess engineering, irrespective of whether it is industrially or environmentally oriented, has an essentially common fundamental basis.

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