Development of self-sustaining phototrophic granular biomass for bioremediation applications

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Natural aquatic biofilms (e.g. periphyton) play a major role in the degradation of conventional pollutants as well as xenobiotics that enter our aquatic systems. The remarkable ability of biofilms to degrade pollutants has been harnessed for purposes such as wastewater treatment. Recent developments in aerobic microbial granulation technology have brought about substantial improvements in biofilm-based remediation processes, offering several advantages such as high biomass retention, rapid biomass settling, high tolerance to toxicity, ability to withstand shock loading and low excess sludge production. We hypothesized that the diverse metabolic machinery and mixed microbial (bacterial, cyanobacterial and microalgal) functions of lotic biofilms could be exploited, if they can be successfully reproduced in the laboratory in the form of granular biomass. Accordingly, a method was developed for the cultivation of phototrophic aerobic microbial granules using bubble column photobioreactors. Mixed inoculum consisting of activated sludge and mixed microalgal cultures was added to column-type bubbled photobioreactors, which were operated in sequential batch mode with 24 h cycle time and 30% volumetric retention. Granulation of biomass was achieved within five weeks. The significance of the work is that it combines the advantages of both aerobic granular sludge and phototrophic biofilms. The bioreactors can be operated without addition of any external organic carbon source, as carbon fixation by the phototrophic elements can support the mixed microbial biomass in the reactor. This granular phototrophic mixed microbial biomass consortium has tremendous applications in environmental biotechnology, which was demonstrated by degrading a toxic model pollutant (phenol).

Keywords: Biofilm, bioremediation, granular biomass, microbial aerobic granules.

BIOFILMS are extensively employed in environmental biotechnological applications such as wastewater treatment. Aerobic granulation (carrier-less biofilm) technology has added a promising aspect to wastewater treatment, though it is yet to achieve significant economic success[1]. Aerobic microbial granules have been demonstrated to be useful in several applications ranging from treatment of domestic wastewater to bioremediation of hazardous chemicals, bioreduction of heavy metals and nutrients like nitrates and sulphates[2–4]. Most processes involving biofilms need inputs of energy, which can be provided in the form of reduced carbon compounds[5–7]. Such requirement for external organic carbon input can make the process cost-intensive. However, the need for organic carbon input can be obviated by integrating photoautotrophic organisms (e.g. microalgae or cyanobacteria) into the biomass and providing light for photosynthesis to take place. The photoautotrophs would fix carbon, part of which could be utilized by the microbial community. The diverse metabolic machinery of the mixed microbial consortium (consisting of bacterial, cyanobacterial and microalgal components) present in such photoautotrophic biomass can be gainfully employed for environmental biotechnological applications.

Phototrophic biofilms are ubiquitous in the aquatic environment, where they exist as complex microbial consortia (e.g. cyanobacterial mat, aquatic biofilms and lotic periphyton) containing several types of phototrophs and heterotrophs. They perform a multitude of ecological functions, including primary production, nutrient cycling, organic matter decomposition, pollutant detoxification, hydrocarbon degradation and biogeochemical cycling[8–11]. Phototrophs fix carbon and nitrogen and produce oxygen as a part of their natural ecological role; the fixed carbon and nitrogen serve as energy source and nutrients for the heterotrophs in the biofilm. The heterotrophs, in turn, mineralize the organic matter, including detritus from dead cells, and provide nutrients for the growth of phototrophs. These processes in microbial mats create a self-sustaining micro niche[12,13].

The biotechnological potential of phototrophic biofilms is generally acknowledged[14,15]. However, their large-scale employment is curtailed by the fact that they need exposure to sunlight. The unidirectional nature of sunlight means that phototrophic biofilms need large area for good irradiance and thereby tend to occupy large footprint, which can be costly in space constrained locations. A simple solution to this problem lies in packaging the

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biofilms into dense granules, such that they can be employed in column-type photobioreactors, which have small footprint. This hybrid technology (aerobic microbial granules and phototrophic biofilms) can overcome the twin problems of high cost of organic carbon input and large land area required for aerobic granulation technology and phototrophic biofilms respectively. It may be noted that aerobic granular sludge technology has added a new dimension to biotechnological application of biofilms due to its superiority in terms of high biomass retention, fast separation of biomass from bulk water and ability to deal with a wide variety of wastes and shock loading conditions[16]. The objective of the present work was to integrate the advantages of the two and recreate, under laboratory conditions, natural phototrophic biofilms in the form of microbial granules, so that they can be employed for biotechnological applications such as pollutant degradation.

Phytoplankton are known to form aggregates[17]. Further, cyanobacteria are regularly seen to aggregate in laboratory cultures[18,19]. The growth of cyanobacteria has been demonstrated in rotating annular reactors[20] and air-lift bubbled reactors similar to those used in aerobic granulation[21,22]. It was, therefore, hypothesized that it would be possible to culture phototrophic granular biofilms consisting of three groups of microbes, viz. bacteria, cyanobacteria and microalgae in bubbled column reactor generally used for development of aerobic granules. This article presents the laboratory-level development of biomass in the form of self-sustaining phototrophic granules (SSPGs) comprising heterotrophic bacteria, cyanobacteria and microalgae in laboratory-scale bioreactors and demonstrates their pollutant degradation capability.

Materials and methods

Reactor setup

Bubbled column reactors made of glass have been used in the present study. Simplified schematic of a single reactor is shown in Figure 1. The reactors were 60 cm in height and 6.3 cm in internal diameter with working volume of 1.7 litres and 30% volumetric retention. All reactors were maintained on a 12 h : 12 h light and dark cycle. The reactors were mounted within a steel chamber enclosing cool fluorescent lights (5 × 40 W) and having reflective coating on the walls. The average photon flux on the walls of the reactors was 120 µmol m^{-2} s^{-1}. The reactors were aerated with two aquarium aerators which provided 1 cm s^{-1} upflow air velocity in the reactors. The entire photobioreactor bank was a closed unit with a door in the front. It was installed in ambient room conditions without any temperature regulation facility.

Biomass and cultures conditions

We theorized that there could possibly be two arrangements of microbial consortia in SSPGs. There could be SSPGs with heterotrophic core and outer layers containing phototrophs or SSPGs having uniformly distributed phototrophs and heterotrophs. Accordingly, the column reactors were operated under two different sets of conditions. In the first experiment, activated sludge (obtained from a wastewater treatment plant) was used as the initial inoculum. It was continuously aerated for two days and then transferred to the reactors (granulation reactor; GR) for developing microbial aerobic granules (MAGs). The reactors were operated as sequencing batch reactors (SBRs), which typically have a feeding phase, followed by mixing phase, reaction phase, settling phase and a decanting phase. We operated the bioreactors with 24 h cycle time and 5 min settling time. Settling, decanting and feeding phase typically took 20 min. Synthetic wastewater (SWW) was used as the feed (Table 1). A mixed microalgal/cyanobacterial consortium, cultured from biofilms developed in a freshwater reservoir was added as supplementary inoculum, one week after the appearance of granular sludge in the reactor. This was to ensure that the granular composition included, apart from heterotrophic bacteria, photoautotrophic components such as microalgae and cyanobacteria. However, no attempt was made to characterize the photoautotrophic components. Once the photoauotrophs were added, supply of organic components in the SWW feed was discontinued and only inorganic components were added to the SWW feeding the reactors. Moreover, two weeks after addition of the

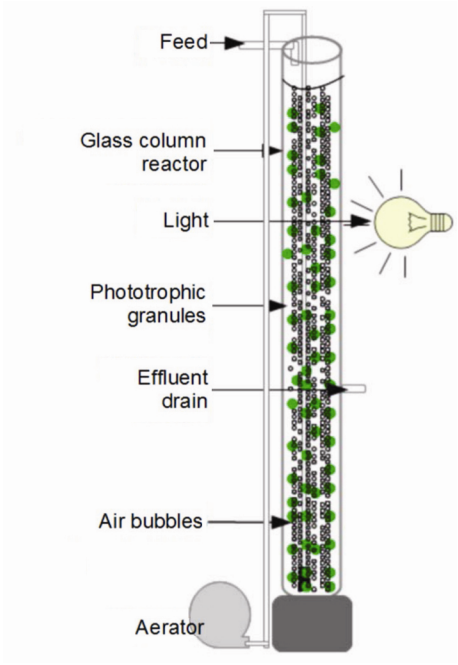

Figure 1. Simplified schematic drawing of bubble column photobioreactors used in the study.

microalgal/cyanobacterial consortium, the settling time was reduced to 3 min. In an SBR, the settling time decides what fraction of the biomass settles and what fraction is removed as part of the discarded water. A short settling time would facilitate selection of granular biomass, while discarding the fluffy slow-settling sludge.

In the second experiment, activated sludge and mixed microalgal/cyanobacterial consortium cultured from biofilms developed in a freshwater reservoir was used as inoculum in column reactors (algal reactor, AR) from beginning of the experiment. The settling time was sequentially reduced from 20 min on the first day to 5 min after one week and then to 3 min after another week. It was further reduced to 2 min, 90 sec, 60 sec and 45 sec after 22, 51, 54 and 96 days of reactor operation respectively. The settling time was reduced in order to select fast settling biomass by rejecting the floccular biomass, which tended to settle slowly. Cycle time was 24 h up to 58 days, after which it was reduced to 12 h. In the case of AR, from beginning of the experiment the reactors were fed with inorganic components of SWW, without addition of any organic carbon source. Table 2 provides the details of operational parameters of the reactors.

Sampling and analysis

Dissolved oxygen (DO), pH and temperature were monitored every 15 min using Hach Multiparameter probe (model HQ40D). Samples of well-mixed bulk solution and effluents were collected twice a week during initial phase of the experiments. Later, the frequency of sampling was reduced to once every five days and then to once every ten days. Settled solids volume (SSV) of all the reactors was recorded on all the sampling days. The samples were used for estimation of mixed liquor suspended solids (MLSS). SSV and MLSS were used for calculating sludge volume index (SVI). All the above analyses were carried out according to standard methods[23]. The biomass was collected for imaging using a Nikon stereo-zoom microscope SMZ-1000 having Olympus DP70 camera, Nikon epifluorescence microscope and Leica SP2-AOBS confocal laser scanning microscope (CLSM). The 488 nm line of an argon laser and 543 nm line of HeNe laser were used to excite the samples under CLSM. Autofluorescence of chlorophyll *a* from cyanobacteria and microalgae was captured at 615 to 705 nm, whereas the autofluorescence of secondary pigments of cyanobacteria was captured at 570–600 nm under CLSM. Crushed granules were stained with acridine orange and observed under epifluorescence microscope. Minimum of ten images per sample (approximately 100 particles) were processed using the ImageJ software of NIH[24] to determine the physical attributes, perimeter and circularity of the granular biomass. The spread of perimeter and circularity was fitted using Gaussian distribution model. Data were analysed and plotted using Microsoft Excel and Origin 8.0 respectively. The data were also statistically analysed for mean, standard error, histogram and correlation. Significance was determined at 95% confidence level.

Table 1. Constituents of synthetic wastewater

Constituents	Concentration (mg l^{-1})
Organic components	
Sodium acetate	320
Citric acid	334
Sodium gluconate	310
Glucose	235
Inorganic components	
$NaH_2PO_4*H_2O$	165
KCl	90
$NaHCO_3$	276.25
$MgSO_4$	142.5
NH_4Cl	107.5

Storage stability of SSPGs

The SSPGs were tested for their storage stability at 25°C in an incubator-cum-shaker without addition of any chemicals and without maintaining any SBR regime. The granules were under continuous shaking at 150 rpm with light intensity of 50 μmol m^{-2} s^{-1}.

Phenol degradation

The ability of the granular biomass developed in the AR rectors to degrade xenobiotics was tested using phenol as a model pollutant. Phenol was selected because of its widespread presence in industrial wastes and its toxic nature. The reactor was inoculated with 700 mg l^{-1} phenol solution at the beginning of each 12 h SBR cycle and bulk samples were collected 0, 1, 2, 3 and 12 h after phenol addition. The collected samples were centrifuged at 10,000 rpm and the supernatant was analysed for phenol concentration by Dionex Ultimate 3000 HPLC system using Waters UV–Vis detector at 270 nm, Waters Nova pack C18 RP column and water : acetonitrile : phosphoric acid :: 79.9 : 20 : 0.1 as mobile phase.

Results

In the first set of experiments, MAGs were developed from the inoculum of activated sludge in bubbled column reactors, which were further inoculated with mixed algal consortium cultured from freshwater. Figure 2 *a–c* shows the image sequence of SSPG formation from activated sludge in the GR reactors. MAGs could be developed after 18 days of SBR operation (Figure 2 *a*). They turned

Table 2. Details of operational parameters of bubbled column reactors

Reactor type	Granulation reactor	Algal reactor
Nutrient media	SWW/SWW (inorganic components) + tap water	SWW (inorganic components) + tap water
Culture/inoculum	Activated sludge leading to granulation + cultured algal consortium from freshwater	Activated sludge + cultured algal consortia from photic biofilm formed in open-water reservoir
Light intensity (μmol m^{-2} s^{-1})	120	120
Light : dark cycle (h)	12 : 12	12 : 12
Aeration (upflow velocity; cm^{-1})	Non-sterile, 1	Non-sterile, 1
Reactor operation	24 h SBR cycle	24 h/12 h (after 58 d) SBR cycle
Working volume (litre)	1.7	1.7
Volumetric retention (%)	30	30
Settling time (initial/later; min)	5/3	20/10/8/5/3/2/1.5/1/0.75

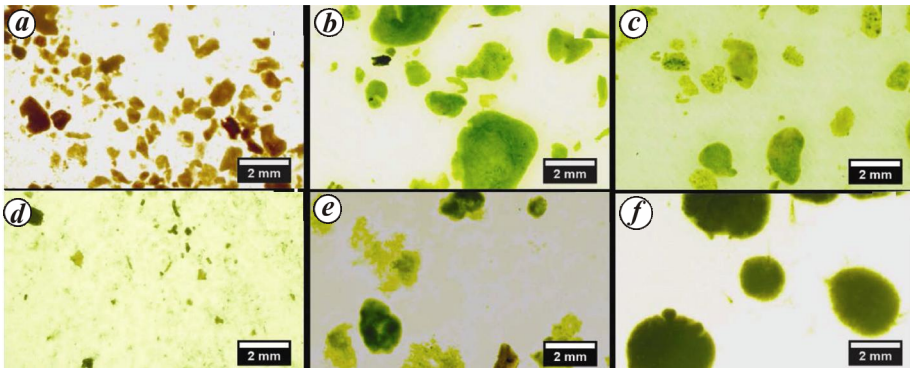

Figure 2. Image sequence showing self-sustaining phototropic granules formation in GR reactor after (***a***) 18 days, (***b***) 33 days, (***c***) 82 days and in the AR reactor after (***d***) 1 day, (***e***) 20 days and (***f***) 96 days of reactor operation.

into green-coloured SSPGs within 9 days of addition of the algal culture. The SSPGs grew in size before breaking up into smaller pieces, which again grew in size (Figure 2 *b* and *c*).

In the second experiment, activated sludge and mixed algal consortia cultured from biofilms developed in a freshwater reservoir were used together as seed inoculum to co-culture bacteria and microalgae in photobioreactors without any carbon source. Figure 2 *d–f* gives the image sequence of development of SSPGs in the AR reactor. Green aggregates could be seen within one day of starting the reactor (Figure 2 *d*), but very little granulation was seen before 20 days of reactor operation (Figure 2 *e*). Thereafter, the sludge in the reactor started turning predominantly granular and became completely granular by 45 days of reactor operation; but it was comprised mostly of very small and elongated particles (75% particles had radius less than 0.8 mm). The small granules were washed off once the SBR cycle time was reduced from 24 h to 12 h. The remaining granules slowly increased in size and circularity. By the end of three months, the sludge was comprised mostly of large granules (Figure 2 *f*).

In both the reactors, the granular sludge settled very fast after cessation of aeration. The granules distributed in full working volume of the reactor up to a height of 55 cm settled to about 8 cm within 1 min of stopping the aeration (Figure 3). Furthermore, the biomass appeared to be energetically supported by the photosynthetic organisms in the granules, as no carbon source was provided in the feed after the addition of algae. Figure 4 *a* shows a single granule from the GR reactor, imaged with CLSM using autofluorescence of chlorophyll *a* and phycobilins. The red-coloured cells are the unicellular cyanobacteria and microalgae, while the yellow-coloured thread-like structures are filamentous cyanobacteria. The filamentous cyanobacteria appear to hold the granule together, providing a kind of internal and external support to the entire granule. The one-month-old granules were crushed and stained with acridine orange to understand the internal structure. As shown in Figure 4 *b* and *c*, bacteria and cyanobacteria could be clearly seen in the crushed granules. The bacterial and cyanobacterial cells can be differentiated by their size and presence of chlorophyll in cyanobacteria. Autofluorescence from their photosynthetic pigments, combined with AO fluorescence,

rendered the cyanobacteria cells orange in colour, while the bacteria can be seen fluorescing green.

Figure 5 describes the physical attributes of SSPGs as a function of reactor operating time. The zero-day values in Figure 5 *a* correspond to bacterial granules after 18 day of SBR operation in the GR reactor. Mean perimeter of granules increased rapidly from 1.7 mm to 4.3 mm 9 days after addition of algal consortium to the reactor. It was observed that granules broke up into smaller particles at this stage and again grew in size with time. Mean circularity of granules appeared to be inversely related to mean perimeter ($r^2 = -0.79$). Figure 5 *b* and *c* gives further details of the sludge structure showing distribution of granule size and circularity as a function of time. More than 30% of granules had a perimeter of 1.11 mm when the algal consortium was added. The SSPGs grew uniformly in size after algal addition and more than 30% and 20% granules were of 2.78 and 4.34 mm perimeter respectively, after 15 days and 27 days of reactor operation. The mode value of circularity of granule decreased with time before increasing again towards the end.

Figure 5 *d* gives the details of physical attributes of granules developed in the AR reactor. After 30 days of reactor operation, reduction in the size of the granules was observed from the initial value of 5 mm, and the mean perimeter reduced to 3.5 mm by 65 days of reactor operation. Thereafter, the mean perimeter of granules continuously increased and reached a relatively high value of more than 25 mm after 127 days of reactor operation. The mean circularity of the aggregates/granules continuously increased from 0.32 after 5 days to 0.58 after 96 days reactor operation. Figure 5 *e* shows the distribution of granules by their size on different days of reactor operation. It can be observed that up to 75 days of reactor operation, the mode value of perimeter of SSPGs was between 2 and 2.9 mm. The mode value of perimeter of granules then increased to more than 8 mm by 116 days of operation. The mode of circularity of granules was about 0.4 till 25 days of reactor operation and it slowly increased to 0.57 by 75 days and 0.68 by 96 days of reactor operation (Figure 5 *f*).

Figure 3. Composite images of reactor showing separation of granular biomass from bulk. Number in each column shows the time (sec) after the stopping of aeration.

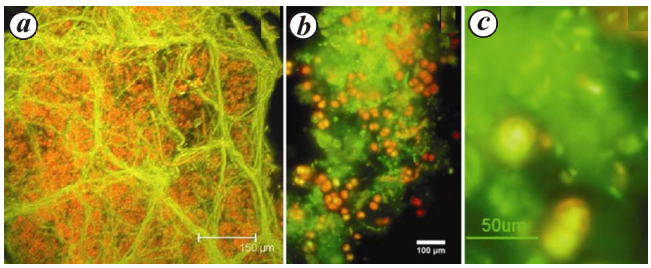

Figure 4. ***a***, Autofluorescence image of SSPG showing filamentous cyanobacteria and microalgae as seen under confocal microscope. Scale bar = 150 µm. ***b***, ***c***, Epifluorescence photomicrographs of crushed SSPG stained with AO showing (***b***) bacteria (green) and (***c***) cyanobacteria (orange-red) under different magnifications.

The temperature, pH and DO of bulk liquid showed diurnal pattern in both GR and AR reactors (Figure 6 *a* and *b*). Both the reactors maintained slightly alkaline pH and highly oxygenated conditions. Dissolved oxygen levels in the GR reactor were inversely related to temperature of the bulk water ($r^2 = -0.79$). The maximum temperature experienced in the AR reactor was lower than that in the GR reactor (Figure 6 *b*). Figure 6 *c* shows SVI of the GR reactor as a function of time. The SVI value of the GR reactor decreased from 247 ml mg^{-1} after 1 day to 33 ml mg^{-1} after 12 days of reactor operation and remained at that level afterwards. In the initial part of study in the AR reactor, when granulation was in progress, SVI increased from 135 to 215 ml mg^{-1}. Once the reactor was primarily granular, SVI started decreasing before reaching 59.3 ml mg^{-1} after 127 days of reactor operation (Figure 6 *d*).

Figure 7 shows the condition of the SSPGs stored at 25°C in an incubator-cum-shaker. As can be seen, no significant physical changes were observed in the SSPGs during the four months of storage. This shows that SSPGs can be generated in large quantities and stored for later use.

The granules developed in the AR reactor were used for studying degradation of a model xenobiotic (phenol). Data of degradation kinetics are presented in Figure 8. Complete degradation of phenol was observed in all the cycles. Moreover, the rate of degradation increased with consecutive cycles, as is evident from increasing slopes of graphs. Heat-killed (autoclaved) biomass did not show any significant reduction in phenol concentration, indicating that phenol removal was not through biosorption (data not shown).

Discussion

The main objective of this work was to combine the advantages of aerobic granular technology and phototrophic

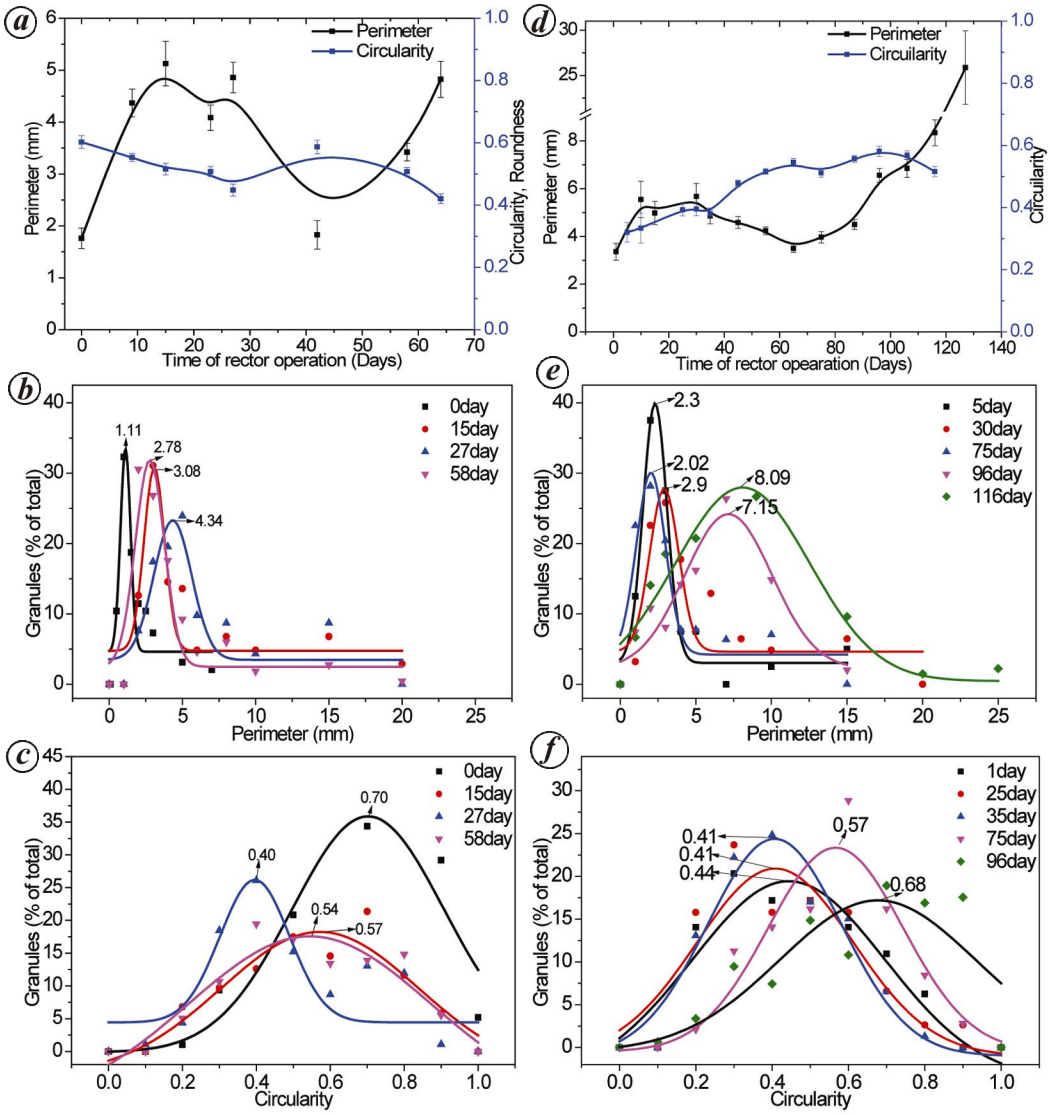

Figure 5. Shape descriptors of granules developed in GR and AR photobioreactors. ***a–f***, Average perimeter and circularity of granules as a function of reactor operation time in GR photobioreactor (***a***) and AR photobioreactor (***d***); distribution of size of granule on different days in GR (***b***) and AR (***e***), and distribution of circularity of granules on different days in GR (***c***) and in AR (***f***). Error bars represent ± SE.

biofilms for creating a consortium biomass in the laboratory that has great potential in environmental biotechnological applications. The ability of phototrophs to fix solar energy along with their relatively large metabolic machinery can complement the well-established benefits of aerobic granular technology and thereby overcome the difficulties in the practical application of phototrophic biofilms for biodegradation. Phototrophs such as cyanobacteria and algae have relatively large genomes compared to bacteria (0.6 versus 4.5 Mb)[25]. Konstantinidis and Tiedje[26] concluded that larger genomes are 'disproportionally rich in secondary metabolism genes'. Many biotechnological applications take advantage of production of secondary metabolites by microorganisms. Therefore, incorporation of microalgae and cyanobacteria into a bacterial consortium will broaden its metabolic landscape and such a phototrophic microbial consortium with large metabolic machinery and the ability to fix its own carbon can be used for various biotechnological applications. Such consortia can be used for more efficient detoxification of organic/inorganic pollutants or removal of nutrients from wastewaters, compared to the individual microorganisms[27]. According Subashchandrabose *et al.*[27], cyanobacteria and microalgae provide oxygen, an electron acceptor required by the heterotrophic bacteria for pollutant degradation. The bacteria, in turn, support the photoautotrophs by providing carbon dioxide for carbon fixation. The fixed carbon can be used by the heterotrophs as primary electron donor, especially when the pollutant to be degraded is a poor substrate for growth.

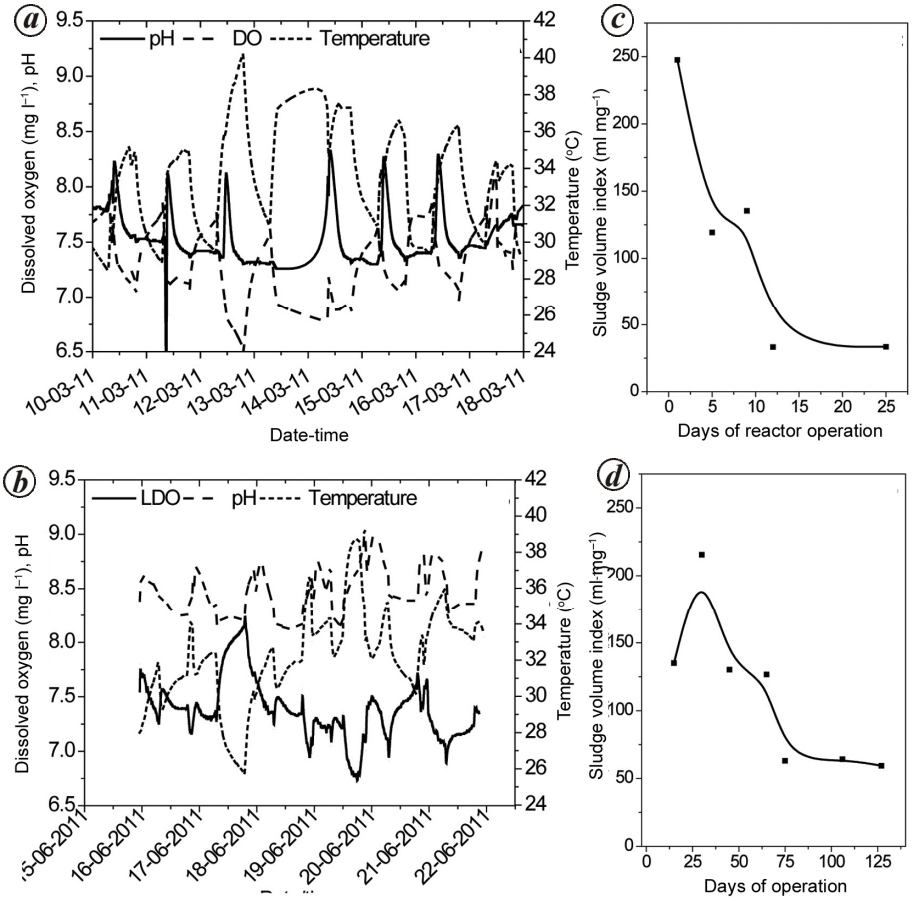

Figure 6. Representative temperature, dissolved oxygen (DO) and pH variation as a function of time in (***a***) GR and (***b***) AR reactors and, (***c***) sludge volume index of GR and (***d***) AR photobioreactors.

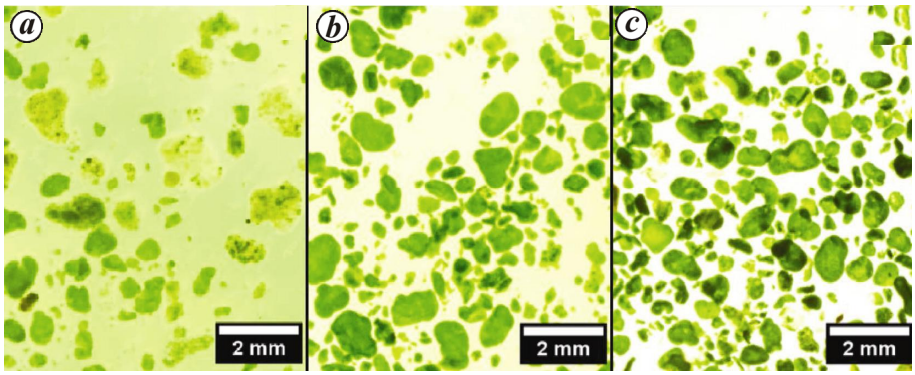

Figure 7. Effect of storage on physical structure of SSPGs. ***a***, SSPGs at the beginning of storage; ***b***, after two months of storage; ***c***, after four months of storage.

Suja *et al.*[28] have highlighted the importance of a labile co-substrate for maintaining the physical and functional integrity of granular sludge, when used for toxic waste degradation.

In the present study, two approaches were adopted for achieving the objective of SSPG development. In the first method, we developed MAGs using activated sludge as the inoculum and added phototrophic organisms at a later stage. In the second method, a mixed inoculum consisting of a mixture of microalgae and cyanobacteria was used along with activated sludge. Activated sludge is almost universally used for developing MAGs, mainly due its high microbial diversity and easy availability.

In the first approach, MAGs quickly developed from the activated sludge under SBR process conditions[29]. MAGs could be developed in a relatively small time (18 days) and thereafter, green granular biomass could be obtained within 9 days of addition of phototrophic

consortium cultured from freshwater. The granules formed in the GR reactors had filamentous cyanobacteria, which helped in packaging the biomass into dense granules. This was reflected not only in fast settling characteristics of the granules, but also in their low SVI. The granules grew into large particles, followed by breaking of the big granules into smaller ones, with subsequent regrowth of the small particles to bigger granules, as has been observed by other researchers[30,31].

These experiments demonstrate the development of SSPGs in bubbled column SBRs using commonly available inoculum sources. But there is a need for supplying organic carbon source during the early stages of development of MAGs. It would be more cost-effective if there was no such requirement of organic carbon addition. Therefore, in our second approach, activated sludge inoculum was mixed with pre-cultured algal/cyanobacterial biomass obtained from biofilms developed in a freshwater reservoir. Since phototrophs were now part of the initial inoculum, the reactor was fed from day one with only inorganic components of SWW. It was demonstrated by Wang *et al.*[32] that gradually increasing selection pressure improves the stability and performance of aerobic granules. In this case, the settling time was reduced from 20 min to 2 min in three weeks of reactor operation and thence to 45 sec by the 96th day. Although the AR reactor showed aggregation of biomass within one day, the sludge became partially granular only after one month of SBR operation and fully granular after 45 days. The granulation process was relatively slow in this case, which could probably be attributed to low selection pressure in the beginning of the experiment. Granulation took place only after the settling time was reduced to 2 min. However, once the sludge became granular, it grew to very large granules with average perimeter of 25 mm or more (diameter = 8 mm). The SSPGs developed in the AR reactors did not break up like those in the GR reactor.

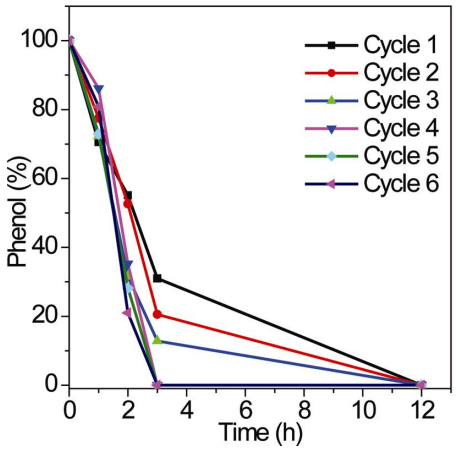

Figure 8. Degradation of phenol by SSPGs in 12 h cycle in sequencing batch reactors. The *y*-axis shows percentage remaining from the starting concentration (700 mg l^{-1}).

This could be due to the fact that the core of SSPGs in the GR reactor was constituted only by bacteria. It is probable that the initial soft core (bacteria plus their EPS) could not sustain the load due to accretion of larger algal cells. In case of the AR reactor, filamentous cyanobacteria (identified as *Plectonema* sp., *Phormidium* sp. and *Oscillatoria* sp.), which were part of the initial inoculum, formed the core of SSPGs, which provided additional strength to the granules. The granules were mostly circular and showed good growth of filamentous cyanobacteria. The very short settling time of 45 sec for a settling distance of more than 35 cm and low SVI showed that the granules were very dense and separated from the bulk really quickly. Furthermore, the reactors were stable till the end of the study period (15 weeks). The bulk water parameters such as DO and pH showed similar trends as observed in other column reactors. The results of this experiment demonstrate the formation of SSPGs without any addition of organic energy sources. Thus, we were able to reproduce the formation of photoautotrophic microbial biomass in the laboratory using a photobioreactor with a small footprint.

Earlier researchers have worked on storage, stability and reuse of microbial granules. It has been shown that MAGs start degrading, if stored without an organic carbon source, even if all other conditions are provided[6]. The SSPGs developed in the present case were stored for more than 8 months (data not shown) in shaking flasks (with illumination) without addition of any organic or inorganic chemicals and without applying the SBR cycle. No significant structural changes were observed in the granules. The growth and functionality of stored granules could be restored when they were transferred to the conditions that existed before storage. Therefore, the SSPGs appear to be more robust than MAGs. Storage for long periods without any addition of nutrients or organic carbon is an attractive feature for large-scale preparation of SSPGs for subsequent use in reactors elsewhere.

The SSPGs developed in the AR reactor were used for demonstration of degradation of phenol, a model xenobiotic compound generally toxic to organisms. The SSPGs quickly adapted to the presence of phenol and up to 700 mg l^{-1} phenol in the influent was degraded within 3 h of addition compared to heat-killed biomass, where no significant reduction in concentration of phenol was observed. The concentration of phenol used here was comparable to that used by other researchers[33]. Quick adaptation and high rate of degradation show that the SSPGs are a potential biomass for cost-effective degradation of toxic wastes.

Conclusion

The present study marks the convergence of two wastewater treatment technologies, i.e. aerobic granular sludge

and phototrophic biofilms. A new type of self-sustaining phototrophic granular biomass consortium was grown using photobioreactors operated in sequencing batch mode. The granules developed were demonstrated for pollutant degradation and could be stored for long periods without loss of structural or functional integrity. This work opens up a new arena in aerobic granular technology and phototrophic biofilms by combining the advantages of both.

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