Bioprocess Biosyst Eng (2010) 33:961–970
DOI 10.1007/s00449-010-0420-z

ORIGINAL PAPER

A BOD monitoring disposable reactor with alginate-entrapped bacteria

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Received: 25 February 2010 / Accepted: 7 March 2010 / Published online: 27 March 2010

Abstract Biochemical oxygen demand (BOD) is a measure of the amount of dissolved oxygen that is required for the biochemical oxidation of the organic compounds in 5 days. New biosensor-based methods have been conducted for a faster determination of BOD. In this study, a mathematical model to evaluate the feasibility of using a BOD sensor, based on disposable alginate-entrapped bacteria, for monitoring BOD in situ was applied. The model considers the influences of alginate bead size and bacterial concentration. The disposable biosensor can be adapted according to specific requirements depending on the organic load contained in the wastewater. Using Klein and Washausen parameter in a Lineweaver–Burk plot, the glucose diffusivity was calculated in 6.4×10^{-10} (m^2/s) for beads of 1 mm in diameter and slight diffusion restrictions were observed ($n = 0.85$). Experimental results showed a correlation ($p < 0.05$) between the respirometric peak and the standard BOD test. The biosensor response was representative of BOD.

Keywords BOD · Biosensor · Alginate bead

List of symbols

Units in the international system

C	Concentration (kg/m^3)
D	Diffusivity (m^2/s)
F	Flux of solution in biosensor (m^3/s)
K_m	Michaelis Menten kinetic constant (kg/m^3)
K_{La}	Volumetric oxygen transfer rate (s^{-1})
M	Mass of beads (kg)
N	Hill coefficient, dimensionless
P	Volumetric fraction of polymer (m^3/m^3)
r	Substrate uptake (kg/s kg)
$\bar{r}$	Observed substrate uptake (kg/s kg)
R	Radius (m)
t	Time (s)
V	Volume (m^3)
X	Biomass concentration (kg/m^3)
Y	Yield (kg/kg)
η	Effectiveness factor, dimensionless
Φ	Thiele modulus, dimensionless

Subindex

H	Hill kinetic
H_2O	Water
L	Liquid
MAX	Maximum
O_2	Oxygen
S	Substrate
SAT	Saturated
X	Biomass

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Introduction

Wastewater comprises liquid waste discharged through domestic residences, commercial properties, industry and/or agriculture and includes a broad range of contaminants and concentrations. Usually, municipal wastewater contains a broad spectrum of contaminants resulting from the mixing of wastewaters from different sources. Biochemical oxygen demand (BOD) is the most commonly used parameter to monitor wastewater of a municipal or industrial discharge. BOD can also be used to evaluate the efficiency of a treatment process, and it is an indirect measure of biodegradable organic compound present in water. BOD has been conventionally determined by taking a sample of water, aerating it to saturate it with oxygen, placing it into a sealed bottle, incubating it in the dark at 20 ±1 °C for a standard period of time and determining the oxygen consumption in the water at the end of the incubation. According to the American standard, the incubation time is 5 days and the BOD values based on this standard are called BOD_5 for short.

Despite the fact that BOD5 has been considered a universal method that needs no expensive equipment and gives a reliable measurement, it is a time-consuming test, unsuitable for process monitoring and control applications. It is therefore of considerable interest to develop alternative test methods for in situ monitoring and on-line control. Several studies have reported biosensing systems that are more specific, sensible, reliable, portable and able to do real-time analysis with simplicity of operation.

Besides providing a fast and reliable measurement of BOD, it is now considered of great interest that the reactor where the cell recognition takes place be disposable and, therefore, easy to replace in the event of culture damage due to the presence of toxic wastes.

A wide range of biosensors for the measurement of available dissolved organic matter have been developed to measure short-term biochemical oxygen demand (BOD) as an estimate of 5-day biochemical oxygen demand in wastewater [1, 2]. A biosensor is a self-contained integrated device, which is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element (biochemical receptor), which is retained in direct spatial contact with a transduction element [3, 4].

The principle of measurement is similar in all BOD biosensors. A culture of aerobic heterotrophic microorganisms is energized with a sample containing a soluble carbon source in the presence of a DO sensor [5, 6] and a measurement of CO_2 produced is also considered [7]. The main differences are in the way the bacterial culture is presented in the form of a steady uniform population. At present, we find biofilms and chemostatic systems. The biofilm-type BOD sensors have several advantages including rapid response, simplicity, compact design, low cost and the possibility of instrumentation for on-line applications. Its typical construction includes a microbial film immobilized between a porous cellulose membrane and a gas-permeable membrane working as the biological recognition biofilm. The microbial cells are placed directly on the surface of an electrode [8–11] or on a porous cellulose membrane through suction or entrapment using gel, polyvinyl alcohol [12, 13] or polycarbamoyl-sulfonate [14, 15]. In addition, bacteria immobilized into porous carriers have been used with advantages [7].

It has been recognized that bacteria survive better for long periods when they are entrapped into a protected surface. Cell entrapment has become an industrial solution in applications such as pharmaceuticals, health care and foods [3, 4]. Several carriers prepared from natural materials have been tested and used by other workers and different entrapment techniques are also being tested.

Entrapment in Ca alginate beads has been frequently used for the immobilization of bacteria [16–20] as it is simple and of low cost. Furthermore, alginate is nontoxic and it may be used safely in the determination of BOD in the industry without releasing toxic compounds into wastewater as pollution [21].

Since the work of Kierstan and Bucke [22], the effect of diffusion of metabolites into the cells within the bead has been recognized. In some cases, it can compromise the viability of the cells, while it generates a diffusion protective barrier in others [23]. This situation creates a difficulty in understanding the bioreactor results, making it a necessary well-defined model in terms of mass transport parameters. Different models have been proposed for immobilized eukaryotic cells [24] and bacteria [18–20, 25], allowing behavior simulation of entrapped cells for different bead size or for reactor design [26].

It has been recognized that inside a bead, oxygen consumption is dependent on bead size [25, 27, 28]. It is a frequent occurrence in the operation of an immobilized bacteria biosensor, that the limiting factor is oxygen, especially when there is an excess of substrate. This weakness can be amended when entrapped bacteria are used in well-aerated microreactors. Miscalculation in the construction of beads can result in hypoxia and unreacted biomass inside the bead generating an error in the measurements of respirometric parameters.

The objective of this research was to apply a mathematical model to evaluate the feasibility of using a BOD sensor based on disposable alginate-entrapped bacteria to monitor BOD in situ. Because the model considers the influences of alginate bead size and bacterial concentration,

the disposable biosensor can be adapted according to specific requirements depending on the organic load contained in the wastewater.

Materials and methods

Disposable reactor with alginate-entrapped bacteria

The disposable reactor is a simple glass column connected with silicone tubing. Figure 1 shows the schematic representation of the whole system. The column (15 cm in height × 2.7 cm in diameter), containing the immobilized cells (20 g) suspended in liquid maintenance medium (40 mL), is aerated at the bottom with air previously filtered through a 0.22-μm EPA membrane using an air pump (10 vvm). Airflow mixes the whole liquid and maintains the oxygen close to saturation. A buffer solution is injected continuously at 1 mL/min using a peristaltic pump (Ismatec, Switzerland). The volume of liquid is continuously pumped with a peristaltic pump. A Clark-type oxygen electrode (Elbau, Germany) is introduced using an O-ring fitted at a distance of 4 cm to the bottom to measure DO in the carrier solution to obtain the baseline. Finally, the change in concentration of DO is related to the metabolic degradation of organic matter in the water by the immobilized bacteria present in the alginate bead.

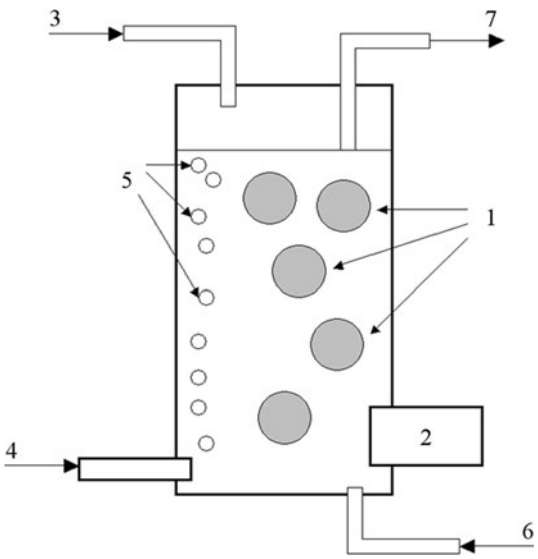

Fig. 1 Diagram of disposable-cartridge biosensor. *1* Beads with bacteria; *2* DO sensor; *3* sample input; *4* continuous air input; *5* air bubbles; *6* continuous saline solution input; *7* continuous solution output

Bacterial selection and identification

Bacteria were isolated from wastewater. Selection was based on their ability to grow fast with colonies appearing frequently in all of the samples. Six samples of wastewater were incubated in TSA agar Petri dishes for 48 h at room temperature. Colonies were selected and incubated in individual vessels with TSB medium. The strain with higher growth velocity (measurement with optic density at 600 nm) was selected to be entrapped into the alginate beads.

For bacterial identification, standard biochemical tests and BIOLOG microplate test panels (Biolog Inc., Hayward, CA, USA) were performed and these were carried out according to the protocol of the manufacturer. The GN2 microplate was incubated for 24 h in the dark at 30 °C. The ability of the microorganism to metabolize different substrates was analyzed and compared with MicroLog2 Biolog data base.

Cell immobilization by entrapment

The bacteria selected were entrapped in calcium alginate beads, using a microencapsulation apparatus with a coaxial superimposed air jet [17, 28]. The commencement solution was 1% sodium alginate (Loba Chemie, Food Grade), decreasing at a constant velocity of 1 mL/min on 50 mM of $CaCl_2$ under gentle stirring [22].

Bacterial concentration in liquid solution was estimated by measuring the optical density at 600 nm against a calibration curve prepared from dry biomass. After washing the beads off with 0.1 M Tris–HCl buffer of pH 7.0, containing 0.5 g/L of 10 mM $CaCl_2$, they were packed into a reactor with approximately 2.1×10^8 cells/bead. After dissolving the beads in sodium citrate, they were estimated by plate count agar techniques.

When not in use, the reactor was kept in 0.1 M Tris–HCl buffer pH 7.0 with 10 mM $CaCl_2$ at 4 °C).

Bacterial distribution inside the alginate bead

Distribution of bacteria inside the alginate bead was explored directly in situ. Fifty beads were submerged into a solution, which contained a working solution of LIVE/DEAD BacLight bacterial viability (0.01 mM of Syto9 and 0.06 mM of propidium iodide). Beads were embedded in a frozen medium (OCT^{TM} Tissue Tek, Sakura Co. Japan) and thick sections of 10 μm were cut on a cryostat [29] at −25 °C. Slices were mounted on silanized slides and kept in the dark at room temperature for 15 min, then observed by epifluorescence microscopy [30].

Glucose diffusion in alginate beads

Alginate beads without cells were introduced in 1% glucose solution and shaken (1,000 rpm) in a thermomixer machine (Eppendorf) at constant temperature (27 °C). Every minute, ten beads were taken out and disintegrated in buffer phosphate (1 M, pH 7) to measure the glucose concentration in the beads. Experimental data was adjusted to an integrated form of second Ficks' law, in analog form as described by Oerther et al. [31] in an alginate sphere with glucose as substrate.

Other methods

Glucose was measured by the spectrophotometric method using a commercial enzymatic kit (glucose oxidase and peroxidase) (Randox Laboratories, UK).

The DO was measured using a DO polarografic sensor (WTW Multiline P4).

For comparison, the biochemical oxygen demand "BOD" was determined according to the standard methods [32].

Results and discussion

Bacterial characterization

Evaluation of the biochemical identification for the chosen population resulted in a probability value of 100% for the isolate being *Enterobacter cloacae*. The biochemical profile is shown in Table 1.

E. cloacae strain grows rapidly and metabolizes 75% of the 95 carbon components present in the Biolog microplates. It is normally present in the human gastrointestinal flora [33, 34] and widely distributed in nature, where it is commonly associated with a variety of plant and animal species, soil and water [35]. This microorganism has a

Table 1 Biochemical profile using GN2 Gram-negative Biolog microplates of bacterial strain

Carbon sources			
Carbohydrates		*Amino acids*	*Polymers*
Dextrin	D,L-α-Glycerol	D-Alanine	Tween 40
Glycogen	Phosphate	L-Alanine	Tween 80
L-Arabinose	α-D-Glucose-1-phosphate	L-Alanylglycine	Glycerol
D-Cellobiose	D-Glucose-6-phosphate	L-Asparagine	
D-Fructose		L-Aspartic acid	
D-Galactose	*Carboxylic acid*	L-Glutamic acid	*Amines and amides*
Gentiobiose		Glycyl-L-aspartic acid	
α-D-Glucose	Acetic acid		N-Acetyl-D Galactosamine
m-Inositol	*Cis*-aconitic acid	Glycyl-L Glutamic	*N*-Acetyl-D Glucosamine
α-D-Lactose	Citric acid	acid	Glucuronamide
Lactulose	Formic acid	L-Histidine	L-Alaninamide
Maltose	D-Galactonic acid	L-Ornithine	
D-Mannitol	Lactone	L-Phenylalanine	*Esters*
D-Mannose	D-Galacturonic acid	L-Proline	
D-Melibiose	D-Gluconic acid	D-Serine	Pyruvic acid methyl ester
D-Psicose	D-Glucuronic acid	L-Serine	Succinic acid mono-methyl-ester
D-Raffinose	β-Hydroxybutyric acid	L-Threonine	
L-Rhamnose	p-Hydroxy phenylacetic	Urocanic acid	
D-Sorbitol	acid	Inosine	
Sucrose	D,L-Lactic acid	Uridine	
D-Trehalose	Malonic acid	Thymidine	
Turanose	D-Saccharic acid		
β-Methyl-D-glucoside	Succinic acid		
	Bromosuccinic acid		
	Succinamic acid		

great capability to adapt to adverse conditions and to develop the ability to degrade new molecules over time [36, 37].

The distribution of bacterial cells inside the alginate beads was explored by the simple method of using frozen sections of beads containing bacteria. Both live and dead bacteria incorporated into alginate beads are shown in Fig. 2. The microbial distribution within the alginate bead was assessed by microscopy analysis. Thin cross sections can be observed in Fig. 2. *Enterobacter cloacae* are distributed over the whole spherical configuration of the bead. They are spread throughout the bead indicating homogeneous distribution. We did not find evidence of bacterial accumulation at the surface or center of the bead. Entrapped bacteria remained alive after 7 days as observed by the predominant green color in the picture.

Oxygen and glucose uptake by entrapped bacteria

Figure 3 represents the glucose uptake by entrapped bacteria at 27 °C. The Lineweaver–Burk plot shows a double slope characteristic for a diffusion-controlled process at a low substrate concentration. On the basis of a Michaelis–Menten kinetic model for glucose, $r_{S/MAX}$ and K_m were estimated for glucose concentrations larger than 10 mg/L where diffusion restriction is low ($r = 0.98$).

$$r_S = r_{S/MAX}\left(\frac{C_S}{K_m + C_S}\right). \quad (1)$$

From here, the following were evaluated:

$r_{S/MAX} = 2{,}000$ (mg/h g-cells)

$K_m = 19$ (mg/L)

Interestingly, the total glucose consumption, fitted well with the Michaelis–Menten kinetics modified by Hill, considering the diffusion restrictions for the alginate bead of 1 mm of diameter (Fig. 4).

$$\overline{r}_S = r_{S/MAX}\left(\frac{C_S^N}{K_H + C_S^N}\right) \quad (2)$$

where $r_{S/MAX} = 2{,}000$ (mg/h g-cells), $K_H = 103$, $N = 1.5$; (with $R = 0.5$ mm).

A linear correlation was also established between glucose consumption for the different initial glucose concentration and oxygen depletion commencing from saturation. We found a highly correlated ($r = 0.97$) behavior, which allowed for the use of a yield factor,

$$Y_{S/O_2} = \frac{r_S}{r_{O_2}} = 12\ (\text{mg/mg}) \approx \frac{1\ \text{mol glucose}}{1/2\ \text{mol } O_2} = 12\frac{1\ \text{mol glucose}}{6\ \text{mol } O_2}. \quad (3)$$

This yield is several times larger than the reported values for bacteria growing in liquid medium ($Y_{X/O_2}/Y_{X/S}$)

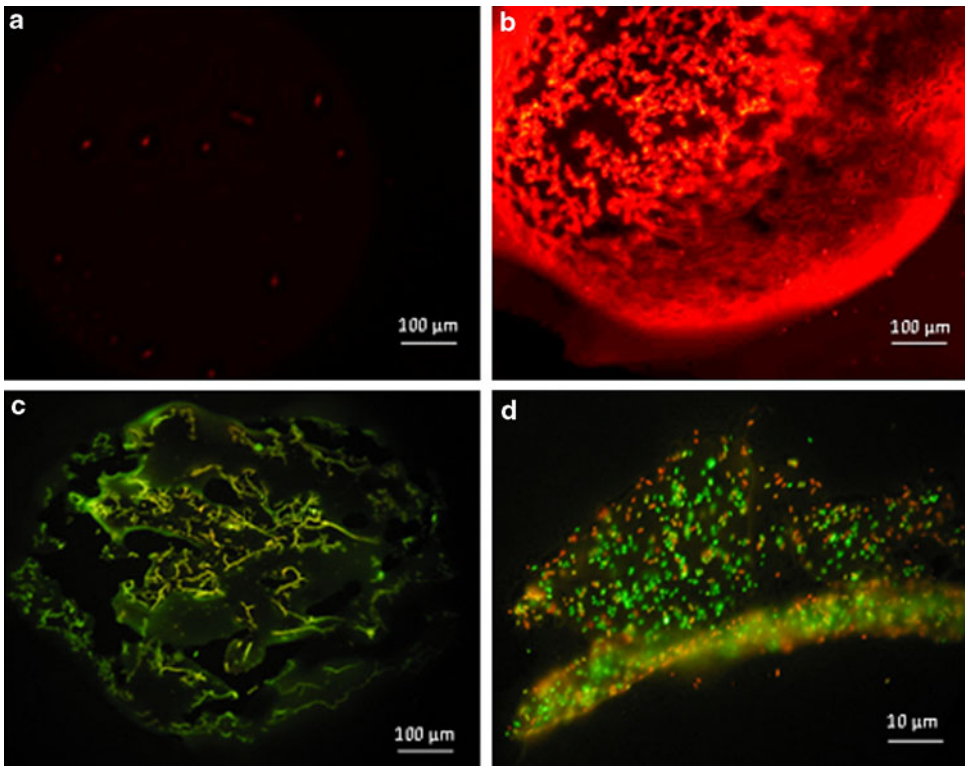

Fig. 2 Viability of microbial cells inside the alginate beads. **a** Background bacteria free alginate bead. **b** Bacterial cells were entrapped in alginate and stained with propidium iodide, magnification 4×. Additionally, slice of bacterial cells entrapped in alginate were stained using Back Light dye indicating that the bacteria remained alive after 7 days of culture. Magnification of 4× (**c**) and 1,000× (**d**)

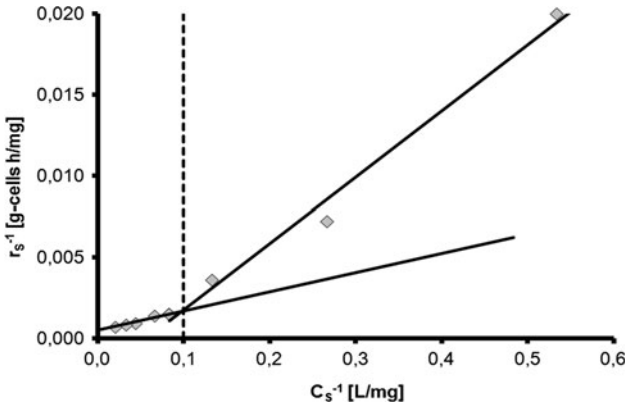

Fig. 3 Lineweaver–Burk plot for glucose consumption by immobilized bacteria

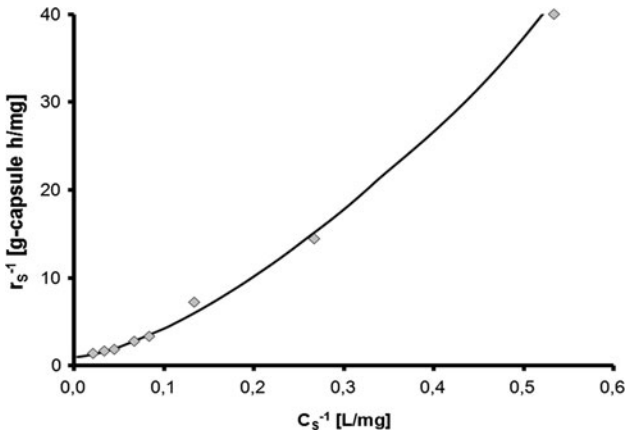

Fig. 4 Glucose consumption by entrapped bacteria and glucose concentration data plotted in reciprocate values indicating in a full line the Hill kinetic (Eq. 2) fit

[38] indicating a large use of substrate mainly for metabolic activities other than energy.

Beads design

Critical oxygen limitation has been reported for all kinds of immobilization systems from enzymes [22] to eukaryotic cells [26]. Recent information gives some insight into the critical diameter at which diffusion limitation becomes important for oxygen and substrates [39]. In our case, for *Enterobacter cloacae* encapsulated into alginate beads, the best approach was to design a proper process: considering a diffusion model for oxygen and glucose, under assumptions of spherical geometry and homogeneous distribution of cells in the capsules:

$$\frac{\partial C_{O_2}}{\partial t} = D_{O_2}\left(\frac{\partial^2 C_{O_2}}{\partial R^2} + \frac{2}{R}\frac{\partial C_{O_2}}{\partial R}\right) - Xr_{O_2}, \tag{4}$$

$$\frac{\partial C_S}{\partial t} = D_S\left(\frac{\partial^2 C_S}{\partial R^2} + \frac{2}{R}\frac{\partial C_S}{\partial R}\right) - Xr_S. \tag{5}$$

The boundary conditions are: symmetry, and glucose and oxygen solubility at the surface of the capsule is proportional to the content of water in the capsule [40].

Oxygen diffusivity was estimated by the Klein and Washausen [41] model:

$$D_{O_2} = D_{O_2-H_2O}e^{(-4P)}. \tag{6}$$

Glucose diffusivity in 1% of the alginate beads was determined experimentally (see "Materials and methods") and was 6.4×10^{-10} (m^2/s). This value is comparatively similar to the diffusion value of glucose in water, 6.9×10^{-10} (m^2/s) [42], and that in alginate (6.8×10^{-10} m^2/s) reported by other authors [43, 44].

Simulation runs (Eqs. 4, 5) show that for the chosen operational conditions, a steady state is reached in a very short time (Fig. 5). In addition, the substrate and oxygen profiles were evaluated showing a flat oxygen profile clearly indicating glucose diffusion control in the process (Fig. 6).

To properly design a bead, taking into account biological reaction inside the matrix together with the diffusion process, the main parameter to consider is the Thiele modulus "Φ" [45] resulting from the dimensionless Eqs. 4 or 5:

$$\Phi = \frac{R}{3}\sqrt{\frac{Xr_{MAX}}{DK_m}} \Rightarrow \Phi^2 = (R^2X)\left(\frac{r_{MAX}}{9DK_m}\right). \tag{7}$$

Since r_{MAX}, D and K_m are constant values for the microorganism and matrix composition, the design variables are radius (R) and bacteria concentration (X), specifically the relationship "R^2X". This module is usually presented in biocatalytic literature associated with the effectiveness factor η (Φ) in the description of immobilized cells [24]. By definition, the effectiveness factor is the ratio of diffusive reaction ($\bar{r}_S$) over non-diffusive reaction (r_S);

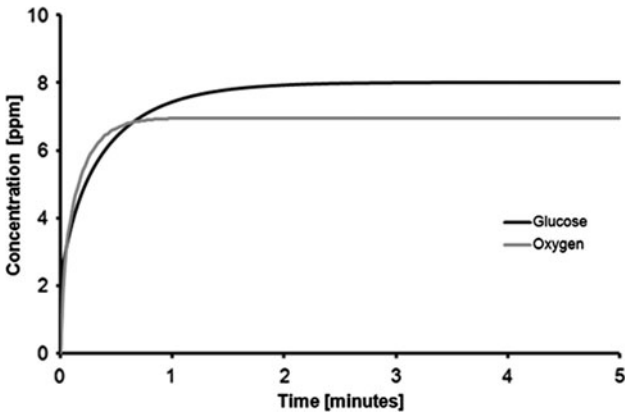

Fig. 5 Simulation of mass transport kinetic in beads with bacteria ($X = 0.5$ g/L; $R = 0.05$ mm)

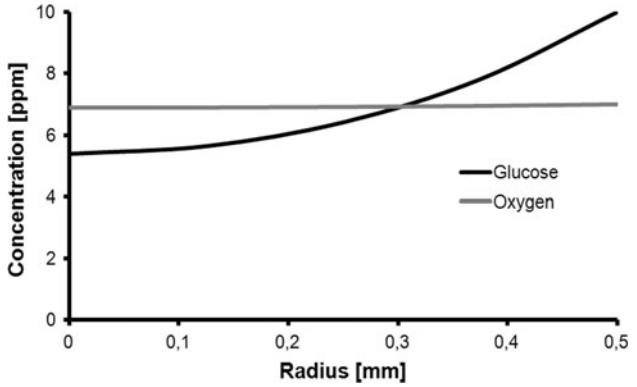

Fig. 6 Prediction of profile concentration inside of alginate beads ($R = 0.05$ mm) with bacteria ($X = 0.5$ g/L) at steady state

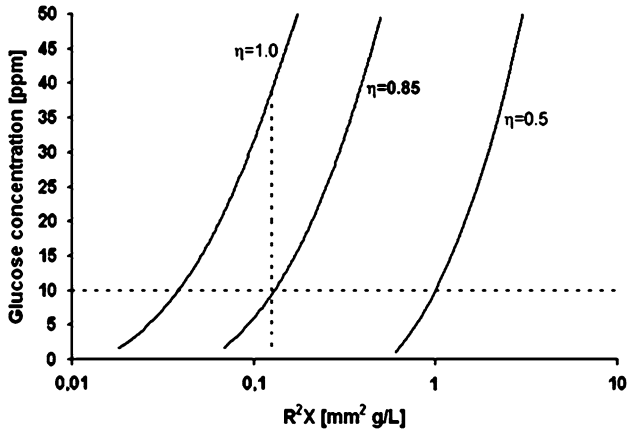

Fig. 7 Map to design entrapment process using *E. cloacae* from wastewater. Segmented line represents the break point in the Lineweaver–Burk plot

therefore, it evaluates the effect of matrix diffusion limitation in the process.

Figure 7 shows the results from the simulation to the general situation. In particular, the break point in the Lineweaver Burk plot ($X = 0.5$ g/L and $R = 0.5$ mm; $R^2X = 0.125$ mm^2 g/L, glucose 10 ppm) as shown in Fig. 3, belongs to the $\eta = 0.85$ curve, clearly indicating slight diffusion restrictions.

Bioreactor–biosensor development

To develop a bioreactor–biosensor prototype for BOD monitoring, 20 g of beads of 1 mm in diameter containing a biomass of 0.5 g/L of *E. cloacae* was introduced into a disposable micro-bioreactor at 27 °C (Fig. 1). The beads were agitated and aerated using airflow of 10 vvm. The volume of the reactor was maintained at 40 mL using a peristaltic pump at 1 mL/min. The DO was monitored online.

Figure 8 shows the changes in the concentration of dissolved oxygen (DO) during a respirometric test, using a

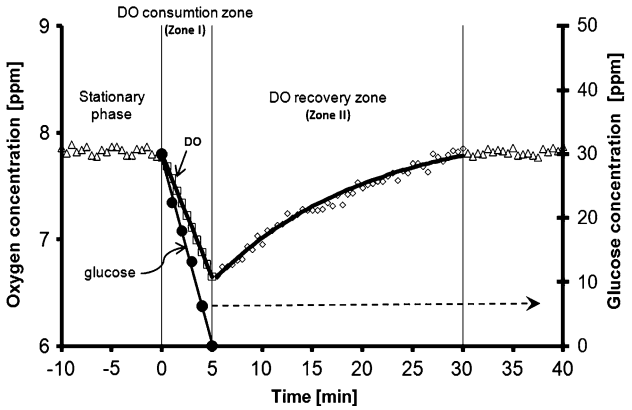

Fig. 8 Zones of respirometric peak by the biosensor

glucose solution as the substrate (1 cm^3 of a 1.2 g/L glucose solution).

We distinguish that:

(a) a conditioning time, where saturation of oxygen is reached, becomes the setting for the baseline (zone 0);
(b) the sample injection, at $t = 0$, followed by oxygen depletion due to the reaction (zone I), as seen by the decrease in DO;
(c) oxygen recovery region (zone II), where the DO increases until a new baseline is obtained.

The time for a full measurement cycle takes about 30 min.

Linear response of the biosensor

To predict BOD, the respirometric peak area was used as the biosensor response. The GGA standard solutions (glutamic acid/glucose) were used to test the linearity using samples of 1 mL. The respirometric peak area was estimated using numerical integration (trapezoidal rule). Figure 9 shows a significant correlation ($p < 0.05$; t test)

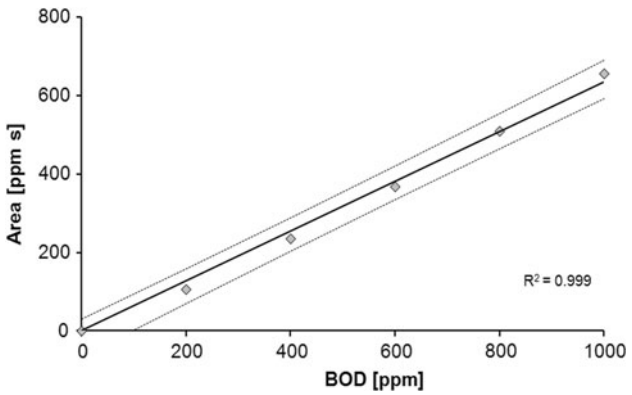

Fig. 9 Correlation between respirometric area and BOD. Showing data (*filled diamond*), linear correlation as a full line together with $p < 0.05$ confidence interval as side lines

between the respirometric peak and the BOD, indicating that the biosensor response is representative of the BOD. Comparable results can be found in literature for a shorter range of BOD [7].

Use of the biosensor in wastewater

Figure 10 shows the response of the biosensor using wastewaters from food industries with BOD ranging between 300 and 1,500 ppm. The comparison of the results obtained by the traditional method [32] and the biosensor showed a good correlation ($r = 0.98$), validating its use as a possible alternative to online method.

Mathematical model of the biosensor

For the biosensor design, a mathematical model was constructed using equations describing a well-agitated tank bioreactor (Eqs. 8, 9). The model considers an open system where the first term correspond to oxygen carried by the continuous flow (input and output of maintenance solution) with a second term related to air transfer of oxygen (air bubbling) and a third term being the oxygen uptake by the bacteria present as a fixed catalyst in the heterogeneous phase (bacterial biomass entrapped in alginate). The right-hand side of the equation corresponds to the oxygen rate of change in the reactor [45].

"Zone I" of the respirometric peak was then modeled by Eq. 8:

$$K_{\mathrm{La}}(C_{\mathrm{O_2/SAT}} - C_{\mathrm{O_2}})V_{\mathrm{L}} + F(C_{\mathrm{O_2/SAT}} - C_{\mathrm{O_2}}) - M\bar{r}_{\mathrm{O_2}} = V_{\mathrm{L}}\frac{\partial C_{\mathrm{O_2}}}{\partial t} \quad (8)$$

and "Zone II" is described by Eq. 9 where the term considering bacteria consumption is neglected due to substrate depletion:

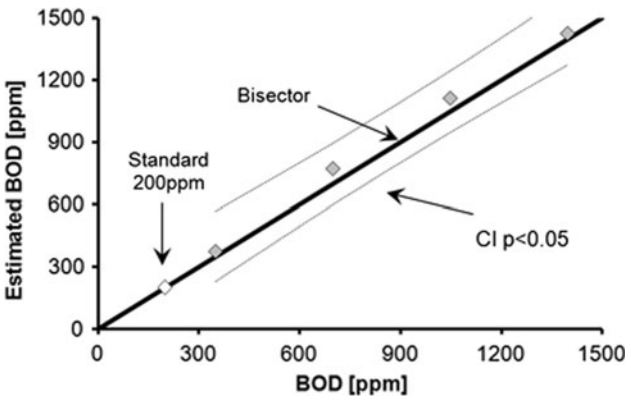

Fig. 10 Comparison between experimental values and predicted values using biosensor in food wastewater. Showing data (*filled diamond*), bisector as a full line together with $p < 0.05$ confidence interval as side lines

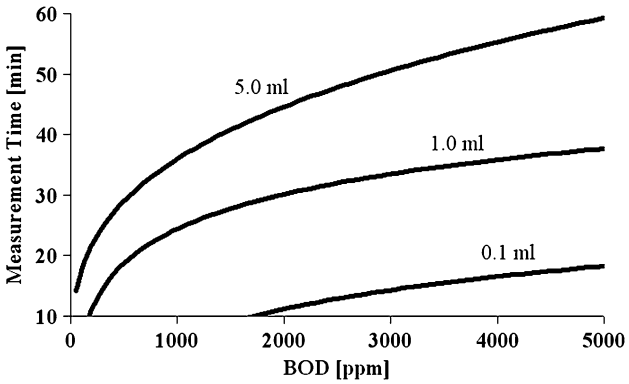

Fig. 11 Simulation of measurement time using three sample sizes (0.1, 1.0 and 5.0 mL) at different BOD ranges

$$K_{\mathrm{La}}(C_{\mathrm{O_2/SAT}} - C_{\mathrm{O_2}})V_{\mathrm{L}} + F(C_{\mathrm{O_2/SAT}} - C_{\mathrm{O_2}}) = V_{\mathrm{L}}\frac{\partial C_{\mathrm{O_2}}}{\partial t}. \quad (9)$$

Global consumption of organic load by entrapped bacteria was calculated with Eq. 2, assuming that a value of BOD yield of 2/3 per substrate, according to ranges reported by Jia et al. [46] using glucose as substrate.

The mathematical model was solved using an explicit finite differential approach. Numerical calculations were made using the software Microsoft Visual basic for Excel. The simulations show that for the chosen design parameters, the time needed for the biosensor to perform one measureable step varies between 10 and 60 min depending on the size of the samples and the BOD organic load (Fig. 11). This result, when extrapolated to a wide range of design variables, shows the great adaptability of the proposed system to any range of BOD with a response time similar to those prototypes of respirometric bioreactor of the last generation [5, 8, 11, 47–50].

Conclusion

A disposal respirometric biosensor, based on disposable alginate-entrapped bacteria, was modeled to detect oxygen consumption. The mathematical simulation presented here considered the influences of alginate bead size and bacterial concentration and its relationship with organic loading of the sample, concluding that beads of 1-mm diameter loaded with 0.5 g of bacteria (dry mass) per liter is an acceptable operational condition, where the diffusion limitation is at a minimum.

Experiments showed that this respirometric bioreactor gave good BOD correlation ($R^2 = 0.99$) with the respirometric area showing a high performance and sensitivity to predict BOD in food wastewater in the range of 200 and 1,500 BOD (ppm) over a short period of time (less than 30 min).

Since alginate entrapment generates a diffusion barrier to nutrients and oxygen, the model can be used to predict the overall behavior and to propose an operating point based on bead diameter and bacterial load where diffusion resistance is minimum. An assumption was made that the cell population was homogeneously distributed, an assumption that was proved correct using microscopy techniques.

Acknowledgments The authors wish to thank CONICYT (FONDEF Grants D00I1091 and D04T2033) and CORFO (FDI-INNOVA Grant 05CR11 PXT-23.).

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