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Studies on phosphate uptake by *Acinetobacter calcoaceticus* under aerobic conditions

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Abstract

Acinetobacter calcoaceticus was cultivated in a well-aerated stirred tank reactor and its phosphate uptake capacity was investigated. Statistical media optimization was done to figure out favourable growth conditions of *Acinetobacter calcoaceticus* NRRLB-552. Plackett–Burman design was used to figure out the key nutrients (sodium acetate, ammonium chloride and calcium chloride) featuring high growth and/or uptake of phosphate. The optimal concentrations for these nutrients were (sodium acetate 5.0 g/l, ammonium chloride 0.67 g/l, calcium chloride 0.05 g/l) obtained by central composite design (CCD) protocols and verified in shake flask cultivations. Predicted and experimental dry cell weights obtained using the optimized media were 2.046 and 2.54 g/l indicating 97% agreement. The optimal values of pH and temperature for growth and phosphate uptake were found to be 7.69 and 31.86 °C, respectively, using CCD. Batch kinetics was also established in shake flask and fermenter using optimized medium and environmental conditions. Phosphate uptakes of 21 mg/g biomass and 36 mg/g biomass were obtained in shake flask and fermenter, respectively. The possible inhibition of nutrients (carbon, nitrogen and phosphate) was also established under shake flask cultivation conditions. Growth of the bacteria was inhibited at a concentration higher than 0.4% carbon and 0.6% nitrogen. However increasing concentration of phosphate did not show any inhibitory effect on growth. The above kinetics and inhibition data will serve as suitable database for the development of a mathematical model for growth and its use will be able to facilitate appropriate reactor design for the removal of phosphates from industrial effluents.

Keywords: Phosphate uptake; *Acinetobacter calcoaceticus*; Aerobic conditions

1. Introduction

Nowadays phosphorous removal is being achieved by means of biological processes rather than chemical treatment methods although its investment costs are higher but annual operating costs are significantly lower, which proves to be more beneficial on long-term basis.

Biological phosphorous removal (BPR) processes are based on the biochemical mechanisms called "luxury uptake" or "overplus accumulation" operated by poly-P bacteria that foresee phosphorous release during anaerobic phase and subsequent phosphorous uptake in excess during aerobic zone. In conventional processes there is involvement of cyclic anaerobic/aerobic phases, where anaerobic phase is needed to produce volatile fatty acids which acts as substrates for phosphate-removing organisms like *Acinetobacter* in activated sludge process under aerobic conditions.

It has also been reported that under anaerobic conditions no phosphate uptake occurs by *Acinetobacter* spp even when fed with acetate [1]. However, phosphorous uptake beyond metabolic needs was detected in aerobic growth of *Acinetobacter* even when no phosphorous was released during the anaerobic phase [1]. Enhanced phosphate uptake in aerobic conditions by *Acinetobacter calcoaceticus* is called as "luxury uptake" that is a larger phosphate uptake by growing bacteria than is strictly necessary for their normal cell metabolism. The total phosphorous content of the cell is

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found to increase in early stages of growth. Polyphosphate accumulation has been studied for various bacteria in activated sludge and it has been found that *Acinetobacter* has maximum capacity to accumulate it intracellularly [2]. They are said to accumulate large amount of polyphosphates of 0.33–0.64 mmol P/g dry cells or 0.9–1.9% of dry cell weight during the logarithmic stage of growth [1]. The phosphate content is reported to vary from 2 to 10% depending on temperature, pH, growth rate and substrate limitation [3,4].

Basic knowledge of growth requirements, kinetic growth coefficients and response to environmental stress is needed with respect to growth and phosphate uptake for this microorganism, for process optimization in biological phosphate removal process. For this purpose, media optimization has been done for the growth *A. calcoaceticus*.

Conventionally fermentations were optimized by carrying out variation of one component at a time. But this approach is time consuming, assumes that the process variables do not interact and that the process response is a function of a single parameter, which is varied. Unlike conventional optimization, statistical optimization methods take into account the interaction of variables in generating the response. One of the statistical designs for the screening of the independent variables was proposed by Plackett–Burman [5]. This had been used in the present study. It is a two factorial design (a series of runs in which combination of two factor levels are included) and offers the screening of a large number of independent factors (N) in small number of experiments ($N+1$). This step is followed by process optimization tool, e.g. response surface methodology (RSM), is most often used to determine the optimum response for a specific range of variable conditions. In this case, the interaction of possible influencing parameters of fermentation can be evaluated with a limited number of planned experiments [6]. A central composite design is usually used to acquire data that will fit an empirical, polynomial model. A central composite experimental design coupled with a polynomial model is a powerful combination that usually provides an adequate representation of most continuous response surfaces over a relatively broad factor domain [7]. The effect of environmental conditions (pH and temperature) on growth and phosphate uptake was also studied and their optimal values were determined using central composite design (CCD). Inhibition studies were conducted for studying the effect of increasing carbon, nitrogen and phosphate concentration on the growth rate of the bacteria. These optimized nutrient and environmental conditions were eventually used to study the kinetics of growth and uptake of phosphate under shake flask and fermenter cultivations.

The above experimental results will provide the needed insight with respect to kinetics and inhibition of cultivation which will facilitate the development of a mathematical model. The model will be able to predict suitable reactor operating strategy for optimal phosphate removal from industrial effluents.

Table 1
Concentration range of variables taken for statistical media optimization

Name	Low level	High level
(A) CH_3COON_a (g/l)	1.00	5.00
(B) NH_4Cl (g/l)	0.30	0.70
(C) KH_2PO_4 (g/l)	0.15	0.20
(D) $MgSO_47H_2O$ (g/l)	0.050	0.100
(E) $CaCl_2{\cdot}2H_2O$ (g/l)	0.020	0.050
(F) Trace metal (g/l)	0.50X	2.00X

$X = 0.000375$ g/l.

2. Materials and methods

2.1. Microorganism

A. calcoaceticus NRRL-B552 was used in the present study. It was maintained on 2TY agar (1% yeast extract, 1% tryptone, 0.5% sodium chloride and 2% agar) slants at 5 °C and subcultured monthly.

2.2. Plackett–Burman design

It is necessary to submit the process to an initial screening design prior to optimization. The methodology of Plackett–Burman is a tool for initial screening, since it makes it possible to pick up the relevant factors from a long list.

The experimental design protocol was developed using Design Expert software (Version 5.0.9) (Stat-Ease Corporation, USA). The influence of six variables (as shown in Table 1) on growth was investigated. Each variable was selected at two concentration levels, a high (+1) and a low (−1) (Table 1) on the basis of literature reports. The experimental plan includes a design of 12 experiments (Table 2), which were done in duplicate and were conducted in 250 ml shake flask containing 50 ml media (pH 7.0). Actively grown log phase cells in 2% Luria Broth (LB) cultivated in Erlenmeyer flask at 30 °C and 150 rpm characterized by optical density (o.d.) 0.35 (dilution factor, $D = 10$) were used as inoculum, and then 5% v/v inoculum was added to media. The experiments were conducted in Erlenmeyer flask incubated in an incubator shaker rotating at 150 rpm for 24 h at 30 °C. The

Table 2
Experimental design given by Plackett–Burman

Experiment	A (g/l)	B (g/l)	C (g/l)	D (g/l)	E (g/l)	F (g/l)	DCW (g/l)
1	0.1	0.3	0.15	0.1	0.05	2.0	1.016
2	0.1	0.7	0.2	0.05	0.05	1.0	0.982
3	0.1	0.7	0.15	0.05	0.02	2.0	1.027
4	0.5	0.3	0.2	0.05	0.02	1.0	2.327
5	0.5	0.3	0.15	0.05	0.05	2.0	2.758
6	0.1	0.7	0.2	0.1	0.02	2.0	1.238
7	0.5	0.7	0.2	0.05	0.05	2.0	3.052
8	0.5	0.7	0.15	0.1	0.02	1.0	2.802
9	0.1	0.3	0.2	0.1	0.05	1.0	1.132
10	0.5	0.3	0.2	0.1	0.02	2.0	2.326
11	0.1	0.3	0.15	0.05	0.02	1.0	1.071
12	0.5	0.7	0.15	0.1	0.05	1.0	2.831

Table 3
Experimental design for response surface methodology

Experiment	CH_3COONa	NH_4Cl	$CaCl_2{\cdot}2H_2O$	Response (DCW)
1	3.00	0.50	0.04	1.693
2	1.00	0.30	0.02	0.814
3	1.00	0.30	0.05	0.816
4	5.00	0.70	0.02	2.563
5	3.00	0.50	0.04	1.8
6	3.00	0.50	0.06	1.829
7	3.00	0.50	0.04	1.79
8	5.00	0.30	0.05	2.318
9	1.00	0.70	0.05	0.806
10	6.36	0.50	0.04	2.676
11	3.00	0.50	0.01	1.569
12	3.00	0.16	0.04	1.657
13	3.00	0.50	0.04	1.539
14	3.00	0.50	0.04	1.545
15	3.00	0.84	0.04	1.701
16	3.00	0.50	0.04	1.536
17	−0.36	0.50	0.04	0.012
18	1.00	0.70	0.02	1.226
19	5.00	0.70	0.05	2.492
20	5.00	0.30	0.02	2.051

response (last column) studied was biomass (dry cell weight (DCW)) (Table 2). These results were analysed by Design Expert software to calculate *t*-values (Table 6) on the basis of which significant factors affecting the response (DCW) were derived. Three components (namely sodium acetate, ammonium chloride, calcium chloride) in the decreasing order of positive *t*-values were selected for further optimization by CCD.

2.3. Response surface methodology (RSM)

Once the relevant factors having high *t*-values were selected by Plackett–Burman design, the RSM was used to determine the optimum concentration of these factors affecting cell growth, rest of the factors being kept at a constant level. CCD developed using Design Expert software (Version 5.0.9, Stat-Ease Corporation, USA) was used to optimize the concentration of the factors selected from Plackett–Burman Design. The design included a set of 20 experiments (Table 3). Response taken was dry cell weight at the end of 24 h. Experiments were conducted in 250 ml Erlenmeyer flask containing 50 ml media (pH 7.0), the rows of Table 3 represented the medium recipe of each experiment. Inoculum used was same as that for Plackett–Burman experiment. The flask was kept in incubator shaker maintained at 30 °C and rotated at 150 rpm. Design Expert software was used to generate model equations and calculate their parameters. Model equations were used to generate contour plots to understand the interaction of various factors. The response surface plots demonstrated the relative effect of two variables on the response when the third factor was kept constant. A special feature of the software, point-prediction, was used to determine the optimum values of the selected factors, seriously affecting the growth.

Table 4
Experimental design for pH and temperature optimization by response surface methodology

Experiment	pH	Temperature (°C)	DCW (g/l)	Final phosphate (g/l)
1	7.00	30.00	2.6122	0.0929
2	7.00	30.00	2.662	0.0969
3	6.00	25.00	0.2571	0.165
4	5.59	30.00	0.8194	0.1456
5	8.41	30.00	2.914	0.09
6	8.00	25.00	2.8542	0.071
7	7.00	22.93	2.646	0.0854
8	7.00	30.00	2.4911	0.0934
9	6.00	35.00	2.806	0.1004
10	8.00	35.00	2.644	0.09112
11	7.00	37.07	1.693	0.141
12	7.00	30.00	2.685	0.0905
13	7.00	30.00	2.708	0.106

2.4. Optimization of environmental parameters (pH and temperature)

Temperature and pH were also optimized for maximum biomass and phosphate uptake using RSM in the similar manner as explained above. The experimental design in this case included 13 experiments (represented by rows of Table 4). Inoculum used was same as for the initial screening experiments done using Plackett–Burman protocol. In this case 50 ml optimized media obtained from the earlier studies (Section 2.3) in 250 ml Erlenmeyer flask was cultivated for 24 h at the respective experimental design conditions of pH and temperature. The results obtained were analysed by Design Expert software protocols to facilitate statistical analysis. As explained above model development, contour plots and point-prediction feature of the software were studied to deduce the optimum conditions of temperature and pH for growth and phosphate uptake.

2.5. Media used

Mineral salt media (MSM) used for shake flask and batch studies on fermenter is as shown in Table 5. It was sterilized in autoclave at 15 psi under 121 °C for 20 min.

2.6. Inoculum preparation for shake flask and fermenter

Cells of one slant were added to 250 ml Erlenmeyer flask containing 50 ml of 2% LB media. The shake flask was grown in incubator shaker maintained at 30 °C and rotating

Table 5
Optimized media composition

CH_3COONa (g/l)	5.0
NH_4Cl (g/l)	0.67
KH_2PO_4 (g/l)	0.175
$CaCl_2{\cdot}2H_2O$ (g/l)	0.05
$MgSO_4{\cdot}7H_2O$ (g/l)	0.075
Trace metal (ml/l) [3]	0.075

at 150 rpm. Actively grown log phase cells of optical density 0.35 ($D = 10$) were used as inoculum for media optimization, pH and temperature optimization and inhibition studies. For studying the kinetics of growth and phosphate uptake under optimized conditions in the Erlenmeyer flask and fermenter the inoculum was prepared in two stages. Preinoculum consisted of actively growing cells obtained in the similar manner as explained above. Cells from preinoculum were transferred to the mineral salt medium (MSM) (optimum pH 7.69) (Table 5), obtained from the optimization studies. This was grown at 31.8 °C, in an incubator shaker rotating at 150 rpm, and 5% v/v inoculum was used for further cultivation studies.

2.7. Substrate inhibition studies for carbon, nitrogen and phosphate on growth

Experiments were performed using optimized media (Table 5), with different concentrations of the inhibitory component. Growth inhibition by carbon was verified varying the carbon (sodium acetate) concentration between 0.25 and 10.0 g/l keeping the other media components at their optimized concentration values. Experiments were carried out in shake flasks and optical density measurements were done at an hourly interval. Similarly inhibition by phosphate (potassium dihydrogen phosphate) was checked between the concentration ranges 0.02 and 0.4 g/l again keeping the other components at their optimized values. Inhibitory effect of nitrogen on growth was studied for concentrations of ammonium chloride ranging between 0.2 and 3.0 g/l.

All experiments were done in duplicate in 500 ml Erlenmeyer flasks containing 100 ml of media (initial pH was set at 7.69 using 2 N NaOH). The flasks were incubated in an incubator shaker maintained at 31.8 °C and rotated at 150 rpm. After 5% inoculum was added to the media, samples were withdrawn at an hourly interval for 10 h. A graph was plotted between $\ln X$–$\ln X_0$ (X_0 being the biomass concentration at zero hour) versus time and from its slope specific growth rate was determined, where X is DCW obtained for that concentration of component whose inhibitory effect on growth was tested at different time interval. Finally from this a plot for specific growth rate versus concentration of the inhibitory component under verification was prepared. Luong model ($\mu_i = \mu_m(1 - P/P_m)^n$) was used to mathematically describe the inhibition kinetics [8] where μ = specific growth rate at any C/N ratio, μ_m = maximum specific growth rate, P = any C/N ratio, P_m = C/N ratio for complete inhibition, n = constant. For determining the values of parameters n and P_m, a graph was plotted between $1 - (\mu_i/\mu_{max})$ and $\ln P$, slope of which yielded "n" and intercept was used to get "P_m".

2.8. Study of growth kinetics using shake flask and fermenter studies

As described above, 5% of inoculum prepared in two stages was added to 200 ml of MSM (Table 5)(pH 7.69) using 2 N NaOH taken in 1 l Erlenmeyer flask. This was grown in shaker incubator maintained at 31.8 °C and rotated at 150 rpm. Samples were withdrawn at various intervals and were analysed for biomass, acetate, phosphate and nitrogen content till stationary phase (indicated by constant DCW or optical density) was reached. Experiments were conducted in duplicate and average values are reported.

Batch studies were done on 3.7 l (working volume 2 l) lab-scale aerobic stirred tank reactor (Bioengineering AG, Switzerland). Media and inoculum were prepared as described above (Sections 2.5 and 2.6). Airflow rate was maintained at 1 vvm; stirrer was driven magnetically at 400 rpm in order to provide adequate mixing without causing surface turbulence. The dissolved oxygen was maintained between 50 and 55% by changing the rpm. Experiment was conducted at constant temperature (31.8 °C) and pH 7.69 (maintained by 2 N NaOH and 2 N HCl). Samples were withdrawn at an interval of 2 h for analysis.

3. Analytical methods

Cell growth was monitored by a Unicon Spectrophotometer 930 (Kontron Instruments, Switzerland) by measuring the absorbance of samples at 600 nm. The Berthelot reaction [9,10] was used for the determination of ammonium ions. Acetate was determined by Nucon 5765 (AIMIL India Pvt. Ltd., New Delhi) gas chromatograph with a flame ionization detector (FID) on a Chromosorb 101 column. Nitrogen was used as carrier gas. The temperatures of detector, injector and oven were set at 200, 195 and 180 °C, respectively. Phosphate was determined by converting it to a reduced form of phosphomolybdate complex of blue color followed by measurement of o.d. at 880 nm (ascorbic acid method) [11]. Polyphosphate content of the bacteria was estimated using ascorbic acid method following digestion of cells for 30 min in 0.5 N H_2SO_4 at 100 °C [12].

4. Results and discussion

4.1. Plackett–Burman design

Table 2 shows the distribution of the factor levels in the experiment and the data for the response (DCW) under study. Based on the values of the response obtained, the data analysis was performed for the effect of the six parameters on growth through t-values as given in Table 6. The factors having positive and higher t-values have more significant effect on the response. It is apparent from the data that sodium acetate, ammonium chloride, magnesium sulphate, calcium chloride and trace metals have positive coefficients hence show positive effect on the growth of *A. calcoaceticus* whereas phosphate did not have a significant effect on growth as indicated by its negative t-value. Maximum value of positive coefficient

Table 6
t-Values obtained by the data analysis of Plackett–Burman design

Factor	*t*-Value
(A) CH_3COONa	13.59
(B) NH_4Cl	1.52
(C) KH_2PO_4	−0.41
(D) $MgSO_4{\cdot}7H_2O$	0.35
(E) $CaCl_2{\cdot}2H_2O$	1.01
(F) Trace metal	0.36

was for sodium acetate, which is well understood by the fact of carbon source requirement for growth. Based on the results of Plackett–Burman, three parameters (sodium acetate, ammonium chloride and calcium chloride) were identified in the decreasing order of positive coefficient values for further optimization studies by RSM.

4.2. Response surface methodology

4.2.1. Media optimization

After the Plackett–Burman design, CCD was used to determine the optimum levels of the parameters for optimization of growth. Using CCD, a total of 20 experiments with different combinations of sodium acetate, ammonium chloride and calcium chloride concentrations were performed (as described in Section 2). The response taken was DCW after 24 h as shown in Table 3. The results were analysed by Design Expert software following linear model equation. The parameters of equation were obtained for growth in terms of most significant factors:

$$\mathrm{DCW} = +1.62 + 0.75\mathrm{CH_3COONa} + 0.085\mathrm{NH_4Cl} + 0.016\mathrm{CaCl_2 \cdot 2H_2O}$$

As can be seen, in the linear model the response was directly proportional to all the factors taken, hence could not give a converging point as it happens in the case of a quadratic or cubic model. Thus for the determination of the optimum operating concentration, the predicted values of maximum response possible from different combinations of the concentrations of the factors taken was studied by a particular feature, "Numerical" of the design expert, it gave 10 solutions for getting maximum response, i.e. DCW. All the 10 solutions with their predicted results were experimentally verified and the combination which gave 95% agreement between predicted and the experimental values of the response was taken as the optimum concentration of the three factors (sodium acetate 5.0 g/l, ammonium chloride 0.67 g/l, calcium chloride 0.05 g/l). Rest of the media components were kept constant at their average values between their respective ranges of concentrations taken.

4.2.2. Environmental condition optimization

Response surface analysis done for optimization of pH and temperature for cumulative maximum DCW and phosphate uptake gave cubic model equations as shown below on the basis of the results obtained from the experimental design of RSM (Table 4):

$$\mathrm{DCW} = +2.63 + 0.48A + 1.51B - 0.35A^2 - 0.20B^2 - 0.69AB + 0.13A^3 - 0.92B^3$$

$$\mathrm{PO_4\ concentration} = +0.096 - 0.033A - 0.042B + 9.525\mathrm{E}{-}03A^2 + 6.225\mathrm{E}{-}03B^2 + 0.021AB + 7.577\mathrm{E}{-}03A^3 + 0.031B^3$$

where A is the pH and B the temperature.

For the determination of optimum operating conditions and analysis of the interaction of factors on growth and maximum phosphate uptake, the response surface was studied in detail, the interacting effect of pH and temperature can be seen through contour plots as shown in Figs. 1 and 2. Responses were also studied for all possible pH and temperature combinations by using a particular feature (point prediction) of the design expert software. It allows the study of the response described by the above equation, using independent variation of one parameter at a time keeping the other factors constant at a particular value. The optimized values for pH and temperature were found to be 7.69 and 31.86 °C for which the predicted values for biomass and final phosphate concentration by the software were 3.14 and 0.072 g/l, respectively. This was experimentally verified and actual biomass and final phosphate concentrations were obtained as 3.06 and 0.077 g/l, respectively.

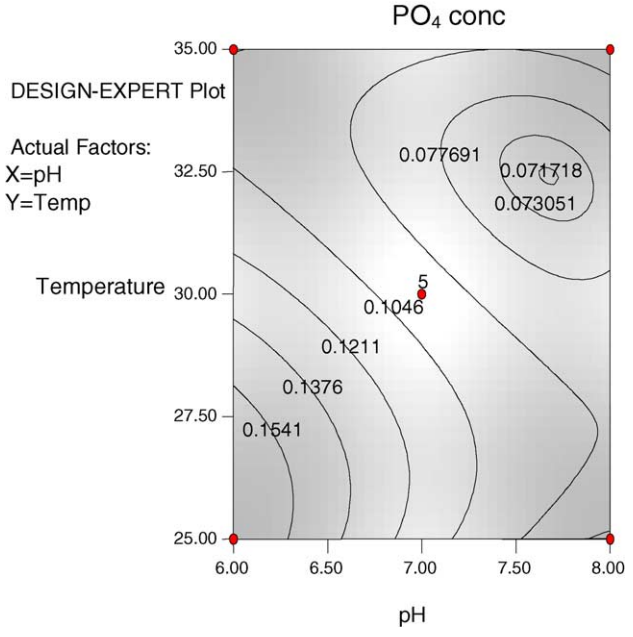

Fig. 1. Contour plot showing combined effect of pH and temperature on phosphate concentration (g/l).

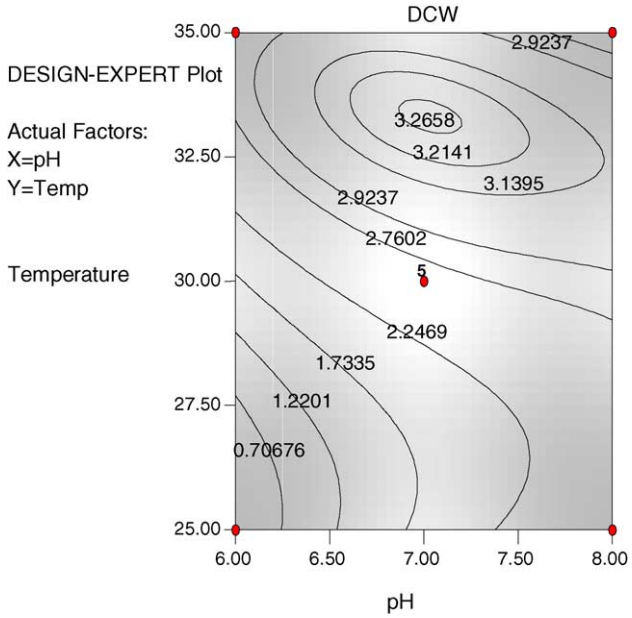

Fig. 2. Contour plot showing combined effect of pH and temperature on DCW (g/l).

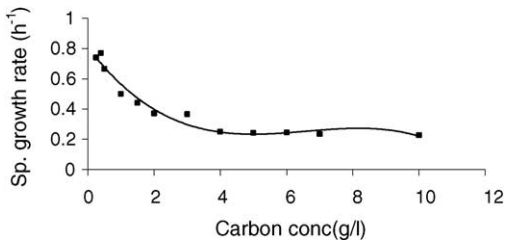

Fig. 3. Plot showing effect of increasing carbon concentration on specific growth rate.

4.3. Substrate inhibition studies for carbon, nitrogen and phosphate on growth

Fig. 3 shows inhibition of growth above 0.4 g/l of carbon, which eventually becomes constant at concentrations higher than 4.0 g/l. From the same data, plot was obtained for specific growth rate versus C/N ratio, as shown in Fig. 4. It is clear from the plot that inhibition begins from 0.6 C/N ratio and specific growth rate becomes constant at ratios higher than 7.0. This plot was used to get the value of parameters $n = 0.3162$ and $(C/N)_m = 30.96$ g/l, ratio at complete inhibition, i.e. where specific growth rate becomes zero.

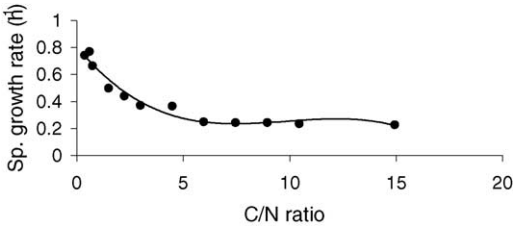

Fig. 4. Plot between specific growth rate vs. C/N ratio.

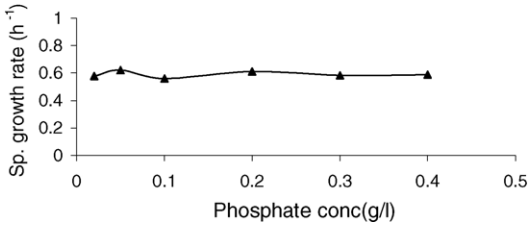

Fig. 5. Plot between specific growth rate vs. phosphate concentration.

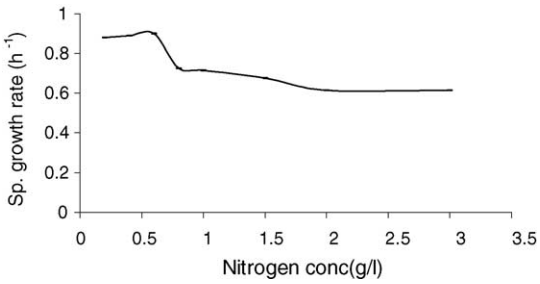

Fig. 6. Plot between specific growth rate vs. nitrogen concentration.

Similarly specific growth rates were determined for different phosphate concentrations and a plot was prepared between them (Fig. 5). It was clear from the graph that increasing concentration of phosphate did not show any inhibitory effect on growth rate as evident from the straight line obtained, almost parallel to *X*-axis.

For nitrogen the plot between specific growth rate and nitrogen concentration is as shown in Fig. 6, which shows that at concentration levels higher than 0.6 g/l, there is growth inhibition, which becomes constant at concentrations above 2 g/l.

4.4. Kinetics of growth in shake flask and fermenter

To study the growth and phosphate uptake kinetics under optimized conditions shake flask cultivation was conducted. The trend of biomass, phosphate uptake and acetate concentrations can be seen in Fig. 7. Maximum biomass obtained was 3.34 g/l with final phosphate concentration of 0.083 g/l towards the end of fermentation, and 3.97 g/l of sodium acetate was consumed out of 5.4 g/l fed initially. The remaining unconsumed acetate may be due to uncontrolled pH conditions in the shake flask. In shake flask 42% of phosphate

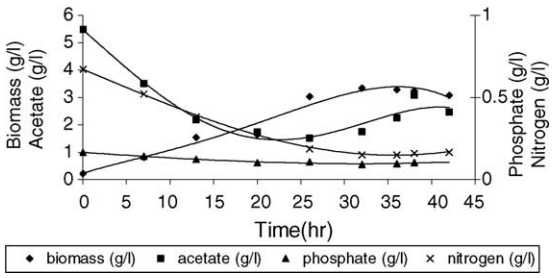

Fig. 7. Nutrient profile in shake flask cultivation.

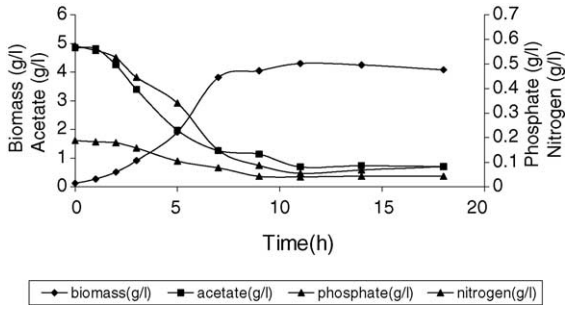

Fig. 8. Nutrient profile obtained in batch reactor.

could be removed, in other words 21 mg of phosphate could be removed per gram of cell.

Batch studies were also conducted on a 3.7 l lab-scale fermenter. The nutrient profile for the cultivation is shown in Fig. 8. Higher amount of biomass (4.08 g/l) was obtained in fermenter resulting in a better acetate uptake upto 4.31 g/l. Phosphate removal upto 78% was achieved in the fermenter, i.e. 36 mg of phosphate could be removed per gram of biomass from the media. Controlled pH and temperature conditions, which is possible only under bioreactor and optimum growth medium, could be the reason for better results in the bioreactor. The result obtained in the present study are comparable to the aerobic batch studies done by Ghigliazza et al. [1] in which a phosphate removal efficiency of 75–80% was obtained and in another such work done by the same authors, including aerobic CSTR resulting in phosphate removal upto 85% has been reported for the culture *A. lwoffi*. Momba and Cloete [3] found that it could remove 34% of the phosphate in activated sludge. However, it has been referred in literature that *A. calcoaceticus* features polyphosphate granules formation under different cultivation conditions [1,4] therefore it will be advantageous to study *A. calcoaceticus* growth in detail. This procedure can avoid the complexity of the delicate process of recirculating the sludge in the presence of alternated anaerobic/aerobic conditions.

5. Conclusion

In order to study enhanced phosphorous removal process in merely aerobic conditions, studies were conducted in an aerated stirred tank reactor inoculated by culture of *A. calcoaceticus* and its phosphate uptake capacity was investigated.

For optimizing the growth of *Acinetobacter* for better phosphate removal statistical media optimization was done using Plackett–Burman and response surface methodology, which yielded optimized media composition as CH_3COONa (5.0 g/l), NH_4Cl (0.67 g/l), KH_2PO_4 (0.175 g/l), $MgSO_4{\cdot}7H_2O$ (0.075 g/l), $CaCl_2{\cdot}2H_2O$ (0.05 g/l), trace (0.075 ml/l).

Optimized environmental conditions of pH and temperature for maximum growth and phosphate removal obtained were 7.69 and 31.86 °C, respectively.

Growth was inhibited at a concentration of carbon greater than 0.4 g/l, nitrogen greater than 0.6 g/l, however only partial inhibition was observed and increasing concentration of phosphate did not show any inhibitory effect on growth.

Shake flask cultivation under optimized conditions resulted in phosphate uptake of 21 mg/g biomass that is 42% phosphate removal while in fermenter the phosphate uptake improved to a value of 36 mg/g biomass hence 78% removal of phosphate was achieved.

Batch kinetic data and inhibition studies conducted in this investigation will be highly useful in the development of mathematical model which can facilitate the design of economic reactor operating strategy for treatment of industrial effluents.

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