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Immobilization of S. flexneri on low cost biopolymer based material for carbon dioxide sequestration

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ABSTRACT

Bacteria was isolated, purified and screened for immobilization on materials. The screened isolate was identified as *Shigella flexneri*. The whole cell of *Shigella flexneri* was immobilized on biopolymer (chitosan-clay and sodium alginate) based material. Optimal immobilization and esterase activity was determined for immobilized cells. Optimal immobilization time was 6h with shaking speed of 160 rpm and 200 rpm for chitosan-clay beads and sodium alginate beads respectively. Immobilization of *Shigella flexneri* on sodium alginate beads is 25% higher than the chitosan-clay beads and may be attributed to increased number of reactive and functional group in sodium alginate beads compared to chitosan-clay beads. Based on p-NPA assay maximum esterase activities was observed to be 135 U/ml for sodium alginate beads with specific activity of 985.44 U/mg and 7.93 U/ml hydratase activity with specific activity of 21.90 U/mg. Similarly for chitosan clay, 127 U/ml esterase activities with specific activity of 520.81 U/mg and 7.21 U/ml hydratase activity with specific activity 20.89 U/mg. Sodium alginate appears to better immobilizing material than chitosan-clay.

KEYWORDS

Chitosan-Clay;
Cell immobilization;
Carbon sequestration;
Shigella;
Bacterial cell.

INTRODUCTION

The rising carbon dioxide (CO_2) emission leading to global climate change is one of the greatest environmental challenges that the world faces today[1]. The temperature of the earth has increase by 0.3°C per decade[2], it is believed that the increase in concentration of CO_2 is responsible for global warming, and is considered to have a significant impact on the earth's climate. Hence it is essential to find ways to reduce the emission of CO_2 to the atmosphere. Various researchers have investigated new approach by using biocatalyst such as carbonic anhydrase (CA) to sequester CO_2 thus anthropogenic CO_2 can be converted into bicarbonate. This process is termed as bio mimetic CO_2 sequestration. Carbonic anhydrase catalyzes the reversible hydration of CO_2 to form a bicarbonate anion and a proton. They are the fastest known enzyme having a high turnover number which makes it a suitable candidate for the conversion of CO_2 to bicarbonates[3-5]. CA is ubiquitous enzyme that catalyzes the inter conversion of carbon dioxide and bicarbonates in an important re-

action in a number of physiological processes including photosynthesis and respiration.

$$CO_2 + H_2O \quad HCO_3^- + H^+ \leftrightarrow$$

There exist a number of problems in the use of enzymes. The immobilization of bacteria is an innovative procedure that can be used to improve the performance and stability of biological treatment systems de-signed for bioremediation of waters contaminated with chlo-rinated solvents (PCE, TCE, etc), hydrocarbons, nitrates, and other biologically degradable compounds[6]. The immobilized cells are capable of dividing in growth medium to form a self-sustaining bacterial monolayer on the patterned areas. The usefulness and efficiency of whole cell immobilization was reported[8-17]. The majority of reported immobilization approaches utilize either nonspecific adsorption of bacterial cells on chemically treated surfaces or physical entrapment of cells in gels[6]. Therefore from the above references, the present study describes the immobilization of bacterial cell *S. flexneri* for their application in the conversion of CO_2 to mineral carbonates by CA activity. The active site of the enzyme present in the cell is responsible for the acceleration of CO_2 hydration as well as for the hydrolysis of esters, therefore esterase activity has been used as a screening tool to determine the activity of CA by using pNPA (para nitrophenyl acetate) as a substrate which gives a yellow product (para nitrophenol) at 348 nm.

Immobilization is an expensive technique; in order to minimize expense immobilizing material being use is to be cheap with good solid support like chitosan. Alginate is commercially available immobilizing material which is a water soluble linear polysaccharide extracted from brown seaweeds. Therefore, the present study is about the comparison of low cost immobilizing material; chitosan with respect to commercially available immobilizing material; sodium alginate. As well as immobilization percentage at different shaking speed and time interval and carbon sequestration by the isolated bacterial extra cellular enzyme, carbonic anhydrase.

SYNTHESIS OF MATERIALS

Chitosan-clay beads

3g chitosan flakes were dissolved in 5% acetic acid (125ml) and stirred 1h then 6g bentonite clay were added and again stirred again for 1h. The mixture was precipitated drop-wise through a pipette, at a constant rate, into a neutralizing solution containing 50% (v/v) NH_3 and stabilizes for 1h. The ammonia solution was prepared by admixing NH_3 solution (25%) with water in 1:1 ratio thus obtaining ammonia solution having molarity 3.2M. The prepared beads were filtered and washed with deionised water until the solution was neutral. They are referred to "wet" composite beads. While they were further dried in oven at 60^0C for 72h, they are referred to "dried" composite beads' used for immobilization.

Sodium alginate beads

4g of sodium alginate was prepared in 100ml distilled water with vigorous stirring for 1h. The solution was precipitated drop-wise through a pipette, at a constant rate, into a neutralizing solution containing $CaCl_2$ (1.725g in 150ml of deionised water). The beads were left in solution for 1h. The prepared beads were filtered and washed with deionised water until the solution was neutral. They are referred to "wet" composite beads. While they were further dried in oven at 60^0C for 72h, they are referred to "dried" composite beads.

Sample collection, isolation of microorganisms

Waste water sample was collected from drainage of NEERI Nagpur Maharashtra (INDIA). Sample was serially diluted to 10^{-7} and plated on nutrient agar. Fifty-one isolates were picked up and purified by repeated streaking on nutrient agar.

Screening and identification of isolates

All isolates are immobilized on chitosan-clay and sodium alginates beads for 6h at 37^0C. The selected isolate was identified based on the morphological, cultural characteristics following growth on HiVeg SS agar media (use for Salmonella, Shigella sp.) and citrate-acetate (CA) medium for rapidly differentiating Shigella (the medium consisted of 3.0 g of sodium citrate, 2.0 g of sodium acetate, 0.2 g of glucose, 1.0 g of dipotassium phosphate, 1.0 g of mono ammonium phosphate, 0.2 g of magnesium sulfate, 5.0 g of sodium chloride, 0.08 g of brom thymol blue, 15.0 g of agar, and 1000 ml of distilled water). Further biochemical characteristics of Shigella were identified by gram staining followed

by 26 different biochemical test using HiMedia biochemical test kit.

Growth curve of isolate

In 100ml of LB broth (Hiveg hydrolysate 10.0g, sodium chloride 10.0g, yeast extract 5.0g and pH 7.5), 100µl of (1.07 O.D) culture was inoculated. Culture was incubated for 0-72h at 37^{0}C with constant shaking speed at 80, 120, 160, 200 and 240 rpms. At the interval of 2h, 4h, 6h, 8h, 10h, 12h, 16h, 20h, 24h, 36h, 48h and 72h of incubation, O.D was measured spectrophotometrically at 600 nm. Growth curve was plotted by using IDBS XLfit 5 software.

Immobilization study of isolate

The protocol for cell immobilization is similar as reported in our previous published work[17]. In short, to 200µl culture of OD 1.15 (at 600nm) in 10ml broth, 0.1g of material was added into tubes and incubated at 37^{0}C for 24h with 2h interval at 80, 120, 160, 200 and 240 rpm. After incubation, supernatant was collected and O.D was measured spectrophotometrically at 600 nm for percent immobilization of whole cell and the beads were collected, washed thoroughly with sterile distilled water. After washing, samples were suspended in phosphate buffer (0.1M, pH 7). The sample was sonicated thrice for 10 s with 30 s intervals followed by centrifugation at 7000rpm for 15min. The supernatant was again centrifuged at 7000rpm for 10min. Similarly the culture (at 600nm, O.D 1.15) without any immobilization matrix was centrifuged and suspended in phosphate buffer (0.1M, pH 7), followed by sonication and centrifugation as described above. The supernatant obtained was used to determine the enzyme activity and protein concentration. The esterase activity of CA in the supernatant was estimated spectrophotometerically at 348nm by measuring the color intensity due to p-NP[21] and protein concentration was determined by Lowry et al. method[22].

Determination of esterase activity

The assay mixture consisting of 1.8 ml phosphate buffer (0.1 M, pH 7.0) and 0.2 ml of enzyme solution (5 mg/ml) or 2ml sample (supernatant) and 1ml of 3mM

TABLE 1 : Cell immobilization and enzyme activity of isolates (mean ± standard deviation, *n* = 3)

S.No.	Isolate	Cell immobilization (%)		Enzyme activity (U/ml)	
		Sodium Alginate	Chitosan-clay	Sodium Alginate	Chitosan-clay
1	IS-1	29.53 ± 0.25	25.56 ± 0.17	55.40 ± 0.26	49.55 ± 0.04
2	IS-3	32.31 ± 0.11	35.45 ± 0.11	60.43 ± 0.15	63.60 ± 0.36
3	IS-7	68.53 ± 0.33	40.71 ± 0.07	98.51 ± 0.13	72.64 ± 0.05
4	IS-9	28.51 ± 0.17	22.64 ± 0.21	53.55 ± 0.13	42.47 ±0.08
5	IS-10	73.51 ± 0.30	48.43 ± 0.14	103.54 ± 0.11	69.30 ± 0.08
6	IS-11	43.48 ± 0.17	40.41 ± 0.20	68.40 ± 0.11	71.61 ± 0.29
7	IS-15	75.35 ± 0.12	40.43 ± 0.14	112.43 ± 0.38	72.58 ± 0.17
8	IS-17	48.57 ± 0.27	48.55 ± 0.06	74.44 ± 0.18	75.51 ± 0.21
9	IS-19	56.44 ± 0.27	50.59 ± 0.19	88.41 ± 0.17	60.32 ± 0.10
10	IS-20	42.61 ± 0.21	40.41 ± 0.10	70.39 ± 0.11	67.58 ± 0.30
11	IS-21	85.40 ± 0.14	59.59 ± 0.07	135.34 ± 0.28	75.40 ± 0.13
12	IS-26	22.48 ± 0.17	29.78 ± 0.06	45.50 ± 0.29	50.38 ± 0.23
13	IS-27	33.55 ± 0.27	47.44 ± 0.13	62.80 ± 0.17	67.43 ± 0.19
14	IS-29	48.43 ± 0.19	42.36 ± 0.07	75.39 ± 0.17	72.50 ± 0.16
15	IS-32	78.39 ± 0.16	42.53 ± 0.12	118.29 ± 0.05	73.48 ± 0.17
16	IS-35	58.45 ± 0.22	42.62 ± 0.03	89.41 ± 0.18	73.50 ± 0.20
17	IS-38	60.52 ± 0.34	41.53 ± 0.06	78.30 ± 0.03	71.63 ± 0.21
18	IS-42	21.47 ± 0.13	19.48 ± 0.09	42.63 ± 0.45	39.45 ± 0.21
19	IS-45	70.30 ± 0.08	49.57 ± 0.04	108.28 ± 0.07	69.35 ± 0.25
20	IS-47	25.30 ± 0.17	21.46 ± 0.03	49.29 ± 0.08	43.61 ± 0.06
21	IS-49	69.38 ± 0.10	48.70 ± 0.12	101.50 ± 0.12	69.37 ± 0.15

paranitro phenyl acetate for its conversion to para nitro phenol. All the experiments like screening, kinetic parameters and carbonate precipitation were repeated twice for better accuracy and blank experiments were also performed throughout the studies.

Determination of hydratase activity

Wilbur–Anderson assay[25] was performed in a vessel maintained at 4°C with water-jacket and constant-temperature circulator by using crushed ice. The vessel was sealed with a rubber-stopper fitted with a pH electrode. A volume, 50 µl sample was added to 3ml of 20mM Tris buffer solution of pH 8.3. The reaction was started by addition of 2ml of water saturated with CO_2 at about 4^0C. CO_2 hydration activity of CA was indicated by the time required for the pH to change from 8.3 to 6.3. The Wilbur–Anderson Units were calculated with equation $(B_{avg}- T_{avg})/ (T_{avg} * \text{Vol. of enzyme})$, and the protein concentration was determined by Lowry et al. method[22] the activity is expressed in Units/mg of protein.

Determination of percent immobilization

Percent immobilization was determined from the difference in esterase activity in the solution before and after the immobilization.

Immobilization yield (%) = (X/A-B) x 100

Where A = added cell, B = free cell, and X = immobilized cell.

Effect of incubation time and shaking speed on immobilized isolate

Study was conducted at different time intervals i.e. 2h, 4h, 6h, 8h, 10, 12, 14, 16, 18, 20, 22 and 24h to determine optimal incubation period for immobilization of isolate on material. Likewise, at different rpm i.e. at 80, 120, 160, 200 and 240 rpm intervals for optimal shaking speed to adsorb cell on materials.

RESULTS AND DISCUSSION

Isolation, screening and identification of microorganism

The two materials based on sodium alginate and chitosan-clay have been synthesised and tested for immobilization of isolates as per protocol mentioned. 51 isolates were purified and tested for immobilization on materials. Out of 51 isolates, 21 isolates were able to properly immobilize on materials. The screening results

TABLE 2 : Biochemical characterization of *Shigella flexneri*

S.No.	Test	Result
1	Gram Staining	Gram negative Straight rods
2	Indole	-
3	Methyl Red	+
4	Voges Proskauer	-
5	Citrate utilization	+
6	Oxidase	-
7	ONPG	+
8	Lysine decarboxylase	+
9	Ornithine decarboxylase	+
10	Urease	-
11	Deamination	-
12	Nitrate Reduction	+
13	H_2S production	-
14	Malonate	+
15	Esculin hydrolysis	+
16	Arabinose	+
17	Xylose	+
18	Adonitol	-
19	Rhamnose	-
20	Cellobiose	-
21	Melibiose	+
22	Saccharose	-
23	Raffinose	-
24	Trehalose	+
25	Glucose	-
26	Lactose	+
27	Mannitole	+

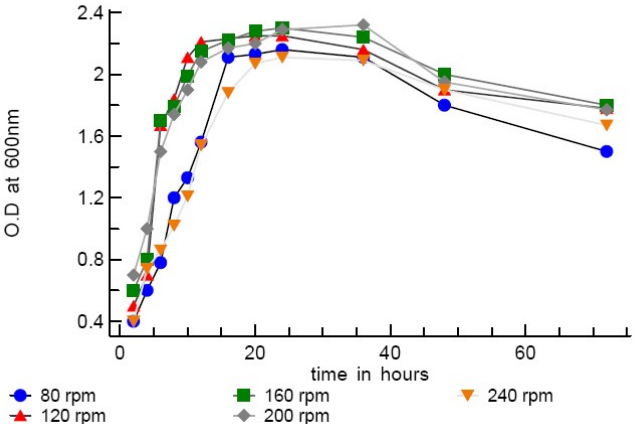

Figure 1 : Growth curve of isolate at different shaking speed

TABLE 3 : Comparison of different shaking speed on immobilization potential (%), enzyme activity (U/ml), protein content (ml/mg) and specific activity (U/mg) and of whole cell *S.flexneri* on sodium alginate beads (mean ± standard deviation, *n* = 3)

Immobilization period	Shaking speed (rpm)				
	80	120	160	200	240
2h					
Cell immobilization (%)	0	59.55 ± 0.14	52.54 ± 0.14	64.38 ± 0.10	64.29 ± 0.08
Enzyme activity (U/ml)	0	70.28 ± 0.09	60.42 ± 0.14	96.36 ± 0.45	96.52 ± 0.28
Protein content (ml/mg)	0	0.368 ± 0.003	0.363 ± 0.004	0.324 ± 0.003	0.322 ± 0.002
Specific activity (U/mg)	0	479.65 ± 0.20	416.67 ± 0.06	750.25 ± 0.24	750.23 ± 0.23
4h					
Cell immobilization (%)	70.42 ± 0.15	68.53 ± 0.31	79.46 ± 0.10	72.43 ± 0.22	72.59 ± 0.20
Enzyme activity (U/ml)	36.27 ± 0.08	45.33 ± 0.06	104.46 ± 0.12	107.46 ± 0.45	107.46 ± 0.21
Protein content (ml/mg)	0.135 ± 0.003	0.158 ± 0.003	0.328 ± 0.003	0.321 ± 0.002	0.322 ± 0.002
Specific activity (U/mg)	673.50 ± 0.21	714.40 ± 0.20	791.67 ± 0.16	835.67 ± 0.26	835.93 ± 0.11
6h					
Cell immobilization (%)	75.40 ± 0.13	78.53 ± 0.15	82.60 ± 0.22	85.41 ± 0.06	79.51 ± 0.23
Enzyme activity (U/ml)	105.27 ± 0.07	100.48 ± 0.18	114.56 ± 0.26	135.56 ± 0.14	121.23 ± 0.15
Protein content (ml/mg)	0.414 ± 0.004	0..337 ± 0.004	0.352 ± 0.01	0.345 ± 0.003	0.358 ± 0.002
Specific activity (U/mg)	639.56 ± 0.10	751.69 ± 0.22	800.31 ± 0.28	985.44 ± 0.19	851.51 ± 0.07
8h					
Cell immobilization (%)	65.44 ± 0.12	65.56 ± 0.32	72.55 ± 0.13	75.50 ± 0.17	75.64 ± 0.17
Enzyme activity (U/ml)	98.32 ± 0.07	98.45 ± 0.24	106.46 ± 0.34	112.53 ± 0.23	112.24 ± 0.19
Protein content (ml/mg)	0.435 ± 0.005	0.405 ± 0.013	0.378 ± 0.003	0.346 ± 0.002	0.343 ± 0.001
Specific activity (U/mg)	569.65 ± 0.34	620.45 ± 0.24	706.45 ± 0.19	817.64 ± 0.11	817.50 ± 0.13
10h					
Cell immobilization (%)	60.17 ± 0.15	55.45 ± 0.21	64.64 ± 0.28	72.55 ± 0.12	72.37 ± 0.38
Enzyme activity (U/ml)	89.41 ± 0.18	66.54 ± 0.27	95.54 ± 0.27	101.45 ± 0.22	101.27 ± 0.10
Protein content (ml/mg)	0.435 ± 0.005	0.396 ± 0.004	0.370 ± 0.002	0.324 ± 0.003	0.328 ± 0.002
Specific activity (U/mg)	517.44 ± 0.17	417.58 ± 0.17	646.37 ± 0.11	773.68 ± 0.19	773.84 ± 0.11
12h					
Cell immobilization (%)	57.30 ± 0.03	51.64 ± 0.20	59.63 ± 0.17	69.53 ± 0.24	69.58 ± 0.23
Enzyme activity (U/ml)	63.36 ± 0.07	59.83 ± 0.16	71.40 ± 0.13	111.57 ± 0.28	111.32 ± 0.15
Protein content (ml/mg)	0.389 ± 0.004	0.421 ± 0.004	0.375 ± 0.004	0.362 ± 0.001	0.365 ± 0.002
Specific activity (U/mg)	408.85 ± 0.09	353.59 ± 0.28	473.43 ± 0.13	765.60 ± 0.19	765.69 ± 0.16
14h					
Cell immobilization (%)	54.28 ± 0.10	51.36 ± 0.14	59.42 ± 0.17	69.39 ± 0.17	62.58 ± 0.15
Enzyme activity (U/ml)	59.22 ± 0.08	58.42 ± 0.18	71.42 ± 0.13	111.26 ± 0.07	98.35 ± 0.09
Protein content (ml/mg)	0.383 ± 0.004	0.417 ± 0.003	0.376 ± 0.003	0.361 ± 0.001	0.323 ± 0.001
Specific activity (U/mg)	387.49 ± 0.09	350.71 ± 0.18	473.56 ± 0.20	765.53 ± 0.18	758.68 ± 0.21
16h					
Cell immobilization (%)	50.26 ± 0.10	49.61 ± 0.22	57.51 ± 0.13	69.36 ± 0.34	59.73 ± 0.09
Enzyme activity (U/ml)	54.26 ± 0.10	55.38 ± 0.10	67.43 ± 0.15	111.16 ± 0.06	90.28 ± 0.06
Protein content (ml/mg)	0.445 ± 0.005	0.430 ± 0.003	0.362 ± 0.003	0.360 ± 0.001	0.310 ± 0.004
Specific activity (U/mg)	303.52 ± 0.18	321.64 ± 0.27	468.65 ± 0.19	765.54 ± 0.27	758.51 ± 0.04
18h					
Cell immobilization (%)	48.72 ± 0.20	48.51 ± 0.19	55.55 ± 0.30	67.56 ± 0.27	57.36 ± 0.11
Enzyme activity (U/ml)	45.22 ± 0.12	51.61 ± 0.22	66.390.13	102.60 ± 0.33	87.43 ± 0.17
Protein content (ml/mg)	0.388 ± 0.003	0.415 ± 0.005	0.361 ± 0.003	0.347 ± 0.005	0.305 ± 0.004
Specific activity (U/mg)	289.73 ± 0.11	311.46 ± 0.27	451.43 ± 0.23	734.36 ± 0.16	724.21 ± 0.12
20h					
Cell immobilization (%)	46.31 ± 0.05	48.50 ± 0.15	55.42 ± 0.18	67.31 ± 0.24	55.48 ± 0.29
Enzyme activity (U/ml)	41.30 ± 0.04	51.52 ± 0.26	66.23 ± 0.17	102.22 ± 0.19	85.36 ± 0.10
Protein content (ml/mg)	0.396 ± 0.004	0.416 ± 0.005	0.358 ± 0.007	0.347 ± 0.001	0.300 ± 0.001
Specific activity (U/mg)	257.84 ± 0.12	311.34 ± 0.16	451.20 ± 0.14	734.19 ± 0.10	710.28 ± 0.07
22h					
Cell immobilization (%)	44.63 ± 0.07	48.22 ± 0.11	51.46 ± 0.23	65.72 ± 0.18	52.60 ± 0.13
Enzyme activity (U/ml)	37.34 ± 0.06	50.55 ± 0.22	58.66 ± 0.25	98.75 ± 0.16	81.48 ± 0.18
Protein content (ml/mg)	0.391 ± 0.002	0.408 ± 0.003	0.354 ± 0.005	0.334 ± 0.003	0.299 ± 0.002
Specific activity (U/mg)	221.57 ± 0.17	284.49 ± 0.20	386.67 ± 0.11	675.74 ± 0.25	675.83 ± 0.10
24h					
Cell immobilization (%)	42.37 ± 0.18	48.00 ± 0.44	51.33 ± 0.29	65.25 ± 0.10	50.34 ± 0.10
Enzyme activity (U/ml)	36.43 ± 0.13	50.53 ± 0.19	58.58 ± 0.21	98.41 ± 0.16	79.51 ± 0.13
Protein content (ml/mg)	0.389 ± 0.002	0.406 ± 0.001	0.352 ± 0.02	0.333 ± 0.001	0.299 ± 0.002
Specific activity (U/mg)	195.73 ± 0.09	287.42 ± 0.40	386.57 ± 0.12	675.62 ± 0.30	654.29 ± 0.10

TABLE 4 : Comparison of different shaking speed on immobilization potential (%), enzyme activity (U/ml), protein content (ml/mg) and specific activity (U/mg) and of whole cell *S.flexneri* on chitosan clay beads (mean ± standard deviation, *n* = 3)

Immobilization period	Shaking speed (rpm)				
	80	120	160	200	240
2h					
Cell immobilization (%)	0	54.29 ± 0.09	48.52 ± 0.15	41.42 ± 0.24	41.13 ± 0.07
Enzyme activity (U/ml)	0	73.46 ± 0.20	25.25 ± 0.27	23.51 ± 0.35	23.54 ± 0.27
Protein content (ml/mg)	0	0.354 ± 0.004	0.258 ± 0.003	0.318 ± 0.003	0.317 ± 0.003
Specific activity (U/mg)	0	521.45 ± 0.16	242.52 ± 0.20	182.71 ± 0.19	182.59 ± 0.06
4h					
Cell immobilization (%)	66.96 ± 0.1	66.74 ± 0.05	59.43 ± 0.25	45.56 ± 0.24	45.10 ± 0.03
Enzyme activity (U/ml)	92.33 ± 0.10	89.48 ± 0.17	78.39 ± 0.19	24.42 ± 0.15	24.30 ± 0.23
Protein content (ml/mg)	0.373 ± 0.003	0.374 ± 0.004	0.266 ± 0.002	0.331 ± 0.001	0.330 ± 0.001
Specific activity (U/mg)	617.36 ± 0.09	597.49 ± 0.16	735.80 ± 0.23	181.71 ± 0.15	181.87 ± 0.04
6h					
Cell immobilization (%)	72.64 ± 0.24	75.38 ± 0.12	82.50 ± 0.15	59.63 ± 0.28	59.33 ± 0.09
Enzyme activity (U/ml)	94.25 ± 0.08	107.51± 0.13	127.60 ± 0.33	75.62 ± 0.20	58.53 ± 0.16
Protein content (ml/mg)	0.391 ± 0.001	0.414 ± 0.005	0.362 ± 0.003	0.362 ± 0.003	0.374 ± 0.002
Specific activity (U/mg)	602.62 ± 0.19	652.52 ± 0.13	881.82 ± 0.26	520.81 ± 0.15	387.28 ± 0.03
8h					
Cell immobilization (%)	65.39 ± 0.11	72.25 ± 0.14	79.51 ± 0.13	52.78 ± 0.12	52.46 ± 0.27
Enzyme activity (U/ml)	82.36 ± 0.09	95.56 ± 0.30	102.66 ± 0.13	69.63 ± 0.20	69.65 ± 0.19
Protein content (ml/mg)	0.373 ± 0.001	0.367 ± 0.003	0.331 ± 0.001	0.328 ± 0.003	0.487 ± 0.001
Specific activity (U/mg)	550.50 ± 0.19	650.79 ± 0.13	772.74 ± 0.21	522.80 ± 0.19	354.47 ± 0.23
10h					
Cell immobilization (%)	60.27 ± 0.18	70.48 ± 0.22	64.50 ± 0.30	49.53 ± 0.31	49.33 ± 0.16
Enzyme activity (U/ml)	76.34 ± 0.09	85.46 ± 0.14	78.52 ± 78.36	32.61 ± 0.21	32.48 ± 0.27
Protein content (ml/mg)	0.367 ± 0.003	0.338 ± 0.003	0.318 ± 0.003	0.316 ± 0.002	0.315 ± 0.001
Specific activity (U/mg)	520.66 ± 0.11	634.52 ± 0.21	619.31 ± 0.26	253.74 ± 0.27	253.96 ± 0.15
12h					
Cell immobilization (%)	51.53 ± 0.20	69.37 ± 0.09	57.44 ± 0.17	45.78 ± 0.05	45.35 ± 0.13
Enzyme activity (U/ml)	55.34 ± 0.07	85.60 ± 0.13	71.51 ± 0.15	30.67 ± 0.20	30.52 ± 0.24
Protein content (ml/mg)	0.537 ± 0.004	0.337 ± 0.003	0.307 ± 0.004	0.304 ± 0.006	0.307 ± 0.011
Specific activity (U/mg)	257.68 ± 0.23	634.39 ± 0.16	581.65 ± 0.25	250.32 ± 0.19	250.17 ± 0.07
14h					
Cell immobilization (%)	48.42 ± 0.19	69.49 ± 0.24	56.37 ± 0.09	44.56 ± 0.28	44.41 ± 0.19
Enzyme activity (U/ml)	37.35 ± 0.06	82.39 ± 0.21	70.41 ± 0.17	29.70 ± 0.34	29.98 ± 0.11
Protein content (ml/mg)	0.451 ± 0.002	0.335 ± 0.006	0.305 ± 0.004	0.308 ± 0.004	0.305 ± 0.007
Specific activity (U/mg)	205.63 ± 0.15	621.45 ± 0.29	581.38 ± 0.29	245.42 ± 0.23	245.22 ± 0.09
16h					
Cell immobilization (%)	43.42 ± 0.17	67.38 ± 0.33	56.63 ± 0.24	44.53 ± 0.22	40.39 ± 0.31
Enzyme activity (U/ml)	30.41 ± 0.14	79.38 ± 0.14	70.33 ± 0.20	29.67 ± 0.44	27.19 ± 0.17
Protein content (ml/mg)	0.433 ± 0.004	0.330 ± 0.002	0.304 ± 0.003	0.306 ± 0.001	0.292 ± 0.001
Specific activity (U/mg)	173.84 ± 0.23	599.68 ± 0.16	581.29 ± 0.18	245.55 ± 0.31	232.14 ± 0.09
18h					
Cell immobilization (%)	40.78 ± 0.12	65.51 ± 0.45	55.33 ± 0.25	43.57 ± 0.26	39.63 ± 0.16
Enzyme activity (U/ml)	24.27 ± 0.07	75.64 ± 0.19	69.55 ± 0.25	29.53 ± 0.22	26.31 ± 0.20
Protein content (ml/mg)	0.377 ± 0.004	0.328 ± 0.003	0.309 ± 0.003	0.303 ± 0.004	0.291 ± 0.002
Specific activity (U/mg)	160.63 ± 0.10	571.49 ± 0.31	565.47 ± 0.24	241.49 ± 0.47	228.83 ± 0.10
20h					
Cell immobilization (%)	37.24 ± 0.10	65.43 ± 0.38	52.53 ± 0.18	42.57 ± 0.25	32.37 ± 0.2
Enzyme activity (U/ml)	19.28 ± 0.05	75.60 ± 0.23	68.50 ± 0.24	29.52 ± 0.20	20.42 ± 0.31
Protein content (ml/mg)	0.308 ± 0.003	0.328 ± 0.003	0.308 ± 0.003	0.310 ± 0.001	0.249 ± 0.001
Specific activity (U/mg)	155.64 ± 0.09	571.47 ± 0.22	555.84 ± 0.27	233.82 ± 0.06	200.83 ± 0.09
22h					
Cell immobilization (%)	34.29 ± 0.08	63.29 ± 0.29	52.42 ± 0.22	42.44 ± 0.37	28.55 ± 0.07
Enzyme activity (U/ml)	17.35 ± 0.07	71.34 ± 0.11	68.44 ± 0.24	29.43 ± 0.10	17.43 ± 0.17
Protein content (ml/mg)	0.295 ± 0.004	0.315 ± 0.005	0.306 ± 0.004	0.308 ± 0.003	0.245 ± 0.001
Specific activity (U/mg)	145.68 ± 0.36	571.38 ± 0.13	555.71 ± 0.59	233.75 ± 0.11	173.74 ± 0.13
24h					
Cell immobilization (%)	32.96 ± 0.14	61.28 ± 0.29	52.38 ± 0.01	42.42 ± 0.53	25.46 ± 0.28
Enzyme activity (U/ml)	16.24 ± 0.09	69.40 ± 0.17	68.51 ± 0.25	29.24 ± 0.15	11.31 ± 0.21
Protein content (ml/mg)	0.291 ± 0.011	0.312 ± 0.004	0.303 ± 0.008	0.305 ± 0.005	0.238 ± 0.001
Specific activity (U/mg)	132.35 ± 0.11	559.37 ± 0.26	548.60 ± 0.25	233.61 ± 0.35	115.17 ± 0.06

of the isolates with their esterase activity respective to percent immobilization are shown in TABLE 1. From all the isolates, seven isolates (IS7, IS10, IS15, IS21, IS32, IS45 and IS49) with their percent immobilization and esterase activity showed good results. However, out of all isolates IS-21 showing the higher esterase activity was selected for this study and further identified.

Isolate IS-21 shows gram negative straight rod shaped cells showing the characteristics of enterobacteriaceae members. For the confirmation of Shigella, isolate was grown on HiVeg SS agar media (use for Salmonella, Shigella sp.) followed by the growth on citrate-acetate medium for rapidly differentiation of Shigella. Further biochemical characteristics of isolate were identified by 26 different test using HiMedia biochemical test kit shown in TABLE 2. Biochemical test results showed Shigella as may be S.flexneri.

Growth curve of S. flexneri at different shaking speed

In order to obtain the growth rate of S. flexneri, 72h growth study was conducted. As Shigella has fastidious growth, lag phase of S. flexneri was found to be from 4h-24h, followed by stationary phase of 24h–36h, thereafter it starts declining (Figure 1).

Percent immobilization, enzyme activity and specific activity of S. flexneri on matrices

The effect of contact time and shaking speed on percent immobilization, enzyme activity in terms esterase activity, and specific activity on chitosan-clay and sodium alginate beads are shown in TABLE 3 and 4, whereas, enzyme activity in terms of hydratase activity is shown in TABLE 4.

Percent immobilization

The optimal contact time at all the shaking speed appears to be 6h, the immobilization thereafter decline. The shaking speed appears to be 160 rpm with 82.5% of immobilization for chitosan-clay beads and 200 rpm with 85.41% of immobilization for sodium alginate beads. The higher decline in immobilization and enzymatic activity for chitosan-clay beads as compared to sodium alginate beads may be attributed to the weaker bonding of the cell with chitosan-clay beads as compared to sodium alginate beads which leads to higher percentage of detachment.

Cell wall of gram negative bacteria is made up of lippopolysaccharide which give negative charge to the bacteria and help in binding with positively charged materials. But due to the presence of clay in chitosan-clay beads material, seem to suppresses the activity of the material for whole cells to adhere. Clay as an admixture does not adsorb whole cell whereas it appears to absorb the enzyme[19]. Thus the optimal immobilization of sodium alginate beads is 25% higher than the chitosan-clay beads.

Enzyme activity on the bases of esterase activity

The enzyme activity of immobilized cell was determined in terms of esterase activity. The esterase activity and specific activity of immobilized cell was found to be of 135 U/ml and 985.44 U/mg respectively at 200 rpm in sodium alginate beads and 127 U/ml and 520.81 U/mg respectively at 160 rpm in chitosan-clay beads.

Enzyme activity on the bases of hydratase activity

The enzyme activity of immobilized cell was also determined following CO_2 hydration assay. The hydratase activity of immobilized beads was 7.21 U/ml and 7.93 U/ml whereas specific activity was found to be 20.89 U/mg and 21.90 U/mg for chitosan-clay beads and sodium alginate beads respectively as compare to free cell with hydratase activity and specific activity of 9.22 U/ml and specific activity of 24.01 U/mg respectively. 4.0

CONCLUSION

In the experimental studies from the above discussions, it may be concluded that sodium alginate beads is better material for immobilization of cells as compared to chitosan-clay beads for all isolates. And out of all the isolates IS-21 i.e S.flexneri is the best isolate showing good immobilization percentage as well as enzyme activity. The optimal contact time at all the shaking speed appears to be 6h, sodium alginate beads show 85.41% of immobilization at 200rpm and chitosan-clay beads show 82.5% of immobilization at 160 rpm .

Enzyme activity and specific activity of immobilized cell on sodium alginate beads increases up to 6h and

thereafter shows a significant decline, the enzyme activity decreases by almost 48% - 56% for different shaking period and may be attributed to detachment of microbes leading to reduced enzyme activity. Similar trend has been observed for chitosan-clay beads i.e. 22% - 75%. The decline in enzymatic activity seems to be more pronounced for chitosan-clay beads as compared to sodium alginate beads at 200 rpm and 240 rpm.

The study primarily demonstrates that the immobilized bacterial strain can provide a better alternative to the existing technology for the sequestration of carbon dioxide.

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