International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 4, 2016

Original Article

UTILIZATION OF ALCOHOLIC BEVERAGE DISTILLERAY WASTE WATER FOR PRODUCTION OF DOCOSAHEXAENOIC ACID

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Received: 16 Nov 2015 Revised and Accepted: 01 Mar 2016

ABSTRACT

Objective: The marine thaustochytrid *Schizochytrium sp* known to produce docosahexaenoic acid (DHA), a polyunsaturated fatty acid with food and pharmaceutical applications. In the present study, distillery waste was studied as an alternative carbon source for DHA production.

Methods: Alcohol beverage distillery wastewater contains significant amounts of proteins and formal-type (free) amino acids, which were used as a raw material for growth of the organism and production of DHA, further effect of carbon, nitrogen, temperature, sea salt initial pH and oxygen transfer rate was studied for DHA production.

Results: In the present study, alcoholic beverage distillery wastewater was used as a medium component, which is one of the major pollutants and of concern to the environmental scientists worldwide. The DHA accumulation in the total lipid was found to be 30 % by using 1 % glucose with the productivity of 55.8 g/l of lipid. The sea salt was optimized to be used at 35 g/l which resulted in best lipid production. Further optimization studies were carried out to improve the biomass yield as well as DHA yield.

Conclusion: In the present research work it was concluded that alterations in various physiochemical conditions like carbon sources, nitrogen sources, temperature, salt concentration, pH showed a major effect on the increase in DHA production This study highlights optimum conditions for increasing DHA production by *Scizochytrium sp.* .The research work gives insight into the rapidly gaining high production of polyunsaturated fatty acids using a cheaper raw material.

Keywords: Distillery waste, Amino acids, Alcoholic beverages, Docosa hexanoic acid

INTRODUCTION

Alcoholic beverage distilleries produce large quantities of wastes, of which composition varies widely according to the raw material distilled. Disposal of distillery wastewater is one of the problems which have to be taken care of, else it can lead to serious environmental threats. Rice, germinated barley, sweet potato or sugarcane is mainly used as raw materials for the production of alcoholic beverages. After the production of distillery drinks the extracted substrate and other materials along with water are left behind which are required to be recycled before disposal. The wastewater has been reported to contain a high concentration of organic matter, which possesses high chemical as well as biological oxygen demand (COD & BOD). The main constituent of distillery wastewater is water which accounts for 90 % (w/w) of the waste, and it can be incinerated resulting in the release of carbon dioxide. A certain portion of such waste is thus incinerated, disposed of in landfills, or dumped into the ocean, thus causing environmental pollution and imbalance, to which little attention has been paid by this industry. Therefore, there is an utmost need for the development of an effective process for the treatment and recycling of distillery waste. The upgrading of the distillery waste can help in many ways to save the natural nutrients of the land and ultimately, the environment.

Distillery wastewater is thought to be biologically safe and nutritionally natural. In Japan, the distillery wastewater from an alcoholic beverage has been effectively utilized for the production of food-related materials [1-7].

Here, our aim was to utilize alcoholic beverage distillery wastewater in the production of polyunsaturated fatty acid using a marine eukaryotic protist (*Schizochytrium* sp.). *Schizochytrium* along with *Thraustochytrium* genus comes under the group thraustochytrids which belong to kingdom Chromista [8, 9]. *Schizochytrium* genus has been known to produce lipids rich in polyunsaturated fatty acids, such as eicosapentaenoic acid (C20:5, n-3), docosapentaenoic acid (C22:5, n-6), and docosahexaenoic acid (C22:6, n-3) [10-2].

Recently DHA, attracted much attention because of its various physiological functions in the human body. The DHA is an essential component of cell membranes in some human tissues and, for instance, accounts for over 60 % of the total fatty acids in the rod outer segment in the retina [13]. Furthermore, DHA is regarded to be essential for the proper visual and neurological development of infants, because of its role as a structural lipid component [14-16]. In addition, the DHA reduces or inhibits risk factors involved in various diseases like cardiovascular [17, 18] and has some positive effects on diseases such as hypertension, arthritis, arteriosclerosis and thrombosis [19].

In this study, the cultural conditions of *Schizochytrium* sp. have been evaluated for the production of polyunsaturated fatty acids, especially DHA and utilization of alcoholic beverage distillery wastewater as a medium component.

MATERIALS AND METHODS

Strain maintenance and seed flask cultivation

M4 medium containing (g/l): glucose (Loba Chemicals, India) 20, Yeast extract (Himedia, India) 2, peptone (Himedia, India) 2, KH_2PO_4 0.25, sea salt (Sigma-Aldrich, India) 35 and distilled water 1, was used for the maintenance of the *Schizochytrium* sp. The cultures were incubated (28 °C/24 h) statically and then preserved at 4 °C until further use.

The seed culture was prepared using M4 medium by scraping the culture from the Petri plates containing statically grown 24 h old *Schizochytrium* sp. The seed flasks were cultivated at 28 °C/220 rpm/24 h. The inoculum size in all cultivations was 10 % (v/v). All medium components except glucose were heat-sterilized (121 °C/20 min/15 lb) separately while glucose was sterilized at 121 °C/15 lb/10 min [20].

Processing of alcoholic distillery wastewater

The distillery wastewater collected from Jagatjit industries, Hamira, Punjab, contained a large amount of mud and unwanted materials which had to be removed before it can be used as a component of media. The wastewater was subjected to centrifugation (2000 rpm/5 min) in a 6 x 25 ml angle rotor in a Sigma 3K30 centrifuge. The pellet obtained was discarded, and the supernatant was filtered with absorbent cotton followed by filtration with Whatman No. 1 filter paper. The Chemical Oxygen Demand (COD), biological oxygen demand, total solids, total nitrogen and formal nitrogen was measured by the standard methods as prescribed in the Indian standards (IS 3025) [21].

Shake flask cultivation for the production of PUFA

Shake flask cultivations (seed cultures, 50 ml M4 medium in 250 ml Erlenmeyer flasks) were carried out (28 °C/pH 7.0/220 rpm/120 h) in a rotary shaker. The seed cultures (5 % of 24 h old) were used to inoculate the production flasks. The pH of the initial medium was adjusted with 1 N NaoH and 1 M HCl using Genei electronic pH meter. The initial studies on optimization of distillery wastewater concentration were done both in batches as well as fed-batch cultivations. The medium used for experiments was designated as M6 which contained (g/l) 20 glucose and 35 sea salt prepared in distillery wastewater. The fed-batch was done with half of the initial concentration of the carbon source used in the medium using the stock solution of the glucose unless otherwise indicated. The fed-batch was done after every 24 h from the inoculation of the culture until the termination of the experiment. The fermentation conditions (28 °C/220 rpm/144 h) used for all the experiments were in M6 medium unless otherwise indicated.

Biomass

The culture broth from each flask was harvested after 144 h by centrifugation (8000 rpm/10 min/20 °C) in a 6 x 25 ml angle rotor in a Sigma 3K 30 centrifuge. The supernatant after separation from pellet was used for the estimation of residual glucose. The pellet was washed for two to three times with sterile seawater and weighed for wet biomass estimation and processed for lipid extraction.

Lipid extraction and analysis

Lipid was extracted by a modified method of [22], using the solvent mixture (ethyl acetate and methanol). The biomass after maceration with methanol was treated with ethyl acetate. The ethyl acetate containing the lipid produced was pooled separately, and anhydrous sodium sulphate was added to remove the excess moisture. The solvent was then filtered through Whatman No. 1 filter paper and evaporated on a rotary evaporator (240 mb pressure/45 °C). The lipid so obtained was subjected to methylation by the procedure as described in previous chapter. The methyl esters of the lipid were analyzed by GC and GCMS conditions being same as previously described.

Optimization of distillery wastewater concentration

This study was aimed to investigate the concentration of distillery wastewater to be used for the production of Docosahexaenoic acid by *Schizochytrium* sp. Optimization of distillery wastewater concentration was done both in batch as well as fed-batch cultivations. Fed-batch was done with half of the initial concentration of the carbon source used in the medium using the stock solution of the glucose. Fed-batch was done after every 24 h from the inoculation of the culture to the day before termination of the experiment. In batch cultivation amount of distillery wastewater used as a component of the medium consisting (g/l) 60 glucose and 35 sea salts were 25 %, 50 %, 75 % and 100 %. The medium was prepared by addition of the above constituents in distilled water. The volume of distilled water (75 %, 50 %, 25 % and 0 %) was used for achieving different concentrations (25 %, 50 %, 75 % and 100 %) of distillery wastewater respectively. The same concentrations of distillery wastewater were used with fed-batch cultivation which consisted of (g/l) (20 glucose and 35 g/l sea salt and distilled water). The glucose (10 g/l) was added after each 24 h.

Time-dependent growth profile studies

The growth profile of *Schizochytrium* sp. in medium containing (g/l) 60 glucose and 35 sea salt in distillery wastewater (M6 medium) was investigated from the time of inoculation of the culture into the production flask until the termination of the fermentation (168 h). The totals of eight flasks were inoculated with seed culture. In every 24 h one flask was harvested and processed for wet biomass, residual glucose, cell count and lipid content. The broth sample was diluted as per the requirement and absorbance (470 nm) was taken (Hitachi, UV/Visible spectrophotometer).

Optimization of carbon source and initial carbon concentration

The distillery wastewater is known to contain many organic as well as inorganic materials. The effect of supplementation of different carbon sources was studied using M6 Medium. All the three carbon sources (monosaccharides, disaccharides, and polysaccharides) were used in a concentration equal to the glucose (60 g/l) concentration used. After each 24 h half the concentration of initial carbon source was used for fed-batch.

The effect of various concentration of glucose, when provided as fed batch in medium containing distillery wastewater, was investigated for the capability of the culture to produce biomass and lipid. The initial concentration of the glucose was kept similar (20 g/l) for all the experimental flasks, but the fed-batch was done after each 24h with different concentrations of glucose (0.5 %, 1 %, 1.5 % and 2 %). Separate stock solutions were prepared for each concentration and keeping the volume of fed-batch similar glucose was added in batches. One flask was cultivated without any carbon source and culture was inoculated in distillery wastewater containing sea salt (35 g/l).

Optimization of sea salt concentration

Effect of different concentrations (0-50 g/l) of sea salt in a combination of distillery wastewater and glucose was investigated for the production of biomass and lipid. Cultivation of *Schizochytrium* sp. was done under fed-batch conditions using glucose 910 g/l) after each 24 h.

Effect of pH, temperature, aeration and agitation

The pH is an important parameter of any fermentation process involving microorganisms. Although the optimum pH for most of the microorganisms falls in between 6-8, hence the investigation was done to see how *Schizochytrium* sp. behaves at different pH in the presence of distillery wastewater. The initial pH of the medium used in this study was 4-9. *Schizochytrium* sp. was cultivated under fed-batch conditions at two different incubation temperatures (20 and 28 °C), to examine the effect of incubation temperature on biomass, lipid, and DHA production. The fermentation was done (5 d) in medium containing (g/l) 20 glucose and 35 sea salt in distillery wastewater.

Effect of aeration was studied in distillery wastewater medium in 1 liter Erlenmeyer flasks. The volume (100, 200 and 300) of the medium in 1 L Erlenmeyer flask was used to achieve decreasing OTR values.

Studies on agitation were carried out at two different rotation speeds (100 and 220 rpm). The fed-batch cultivation was carried out in medium containing (g/l) glucose 20 and sea salt 35 in distillery wastewater (28 °C/120 h).

Residual glucose and optical density measurements

Residual glucose was measured by Collins ferricyanide method by titrating the sample against potassium ferricyanide and NaoH, with methylene blue as indicator. The residual sugar was calculated as mentioned earlier.

The optical density (OD) was measured at 470 nm (Hitachi, UV/Visible Spectrophotometer, Japan).

RESULTS

Alcoholic beverage distillery wastewater, when processed for the estimation of chemical oxygen demand (COD), yielded a value of 20,000 mg/l, while COD of total waste without any processing gave a value of 46,700 mg/l. The pH of the wastewater was acidic (3.7-3.4) in nature. The total nitrogen and other organic and inorganic solids found in the distillery wastewater have been summarized in table 1.

Table 1: **Composition of alcoholic beverage wastewater used in this study**

Parameter	Total waste	Wastewater
pH	3.7±4.4	3.4±0.0
Total nitrogen (%)	0.70±1.11	0.51±0.0
Crude protein (%)	4.18±0.0	2.77±0.0
Formol nitrogen (%)	0.19±2.7	0.26±0.0
Total organic solids	0.20±2.7	0.11±0.0
Total inorganic solids	0.19±2.7	0.13±0.0
COD mg/l	46,700±0.0	20,000±0.0
BOD (mg/l)	30,200±0.0	13,000±0.0

Number of replicates for experiment n=3, Data given in mean±SD

In this study, the measurement of BOD value with increasing incubation period was carried out. It was found that the increase in the BOD value during the first 24 h of incubation was very high (9500 mg/O_2/l), which later increased in such a manner so that the BOD value of next day was lesser than the previous day.

Growth curves studies

Utilization of the distillery wastewater by *Schizochytrium* sp. and its capabilities for biomass, lipid and DHA production was examined. The growth studies were performed by monitoring the optical density, biomass, cell count and lipid content. When distillery wastewater was used instead of peptone and yeast extract in the cultivation medium, it was observed that the initial lag phase was extended and continued until 10-12 h and after which a log phase began where an OD (4.2) was observed at the end of 72 h. The log phase was followed by the stationary phase, and a slight increase in OD (5.0) was recorded at the end of 120 h. The incubation when further extended to 144 h the OD also increased and thereafter a decrease in the O. D (4.8) was observed at 168 h.

Biomass production of the *Schizochytrium* sp. was comparable with the growth pattern as observed during the OD studies. A biomass (46.8 g/l) was obtained at the 72 h, which later increased (57 g/l) at the end of 144 h incubation time. The biomass harvested after 168 h was similar to that obtained at 144 h.

The increase in the number of cells/ml was observed until 72 h, after which it remained constant, for the rest of the incubation period, due to increase in the size The number of cells (8.6 x 10^7) at 168 h, was lesser than that observed (1.3 x 10^8) at 144 h.

During cultivation with distillery wastewater, the cell size increased from 6-7 µ at 24 h to 16 µ at the end of 144 h. The details of the cell size and cell count along with biomass and lipid has been provided in the fig. 1.

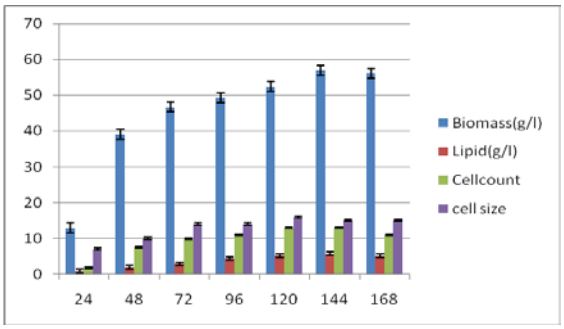

Fig. 1: Cell Size and Cell count along with biomass and lipid Data are given in mean±SD

The fatty acid profile of the *Schizochytrium* sp. is presented in table 2. It was found two PUFA's were present as major fatty acids one being docosahexaenoic acid and the other docosapentaenoic acid. All other fatty acids contributed very less amounts in the total lipid produced by the culture. The 16 carbon long saturated palimitic acid was the major fatty acid with 54.2 %, after which there was DHA and then DPA (table 2).

Table 2: Fatty acid profile of *Schizochytrium* sp. grown on medium containing distillery wastewater

Fatty acids (Carbon no.:no. of double bonds)	Fatty acid	Fatty acid in lipid (% w/w)
14:0	Myristic acid	0.4±5.5
16:0	Palmitic acid	54.2±7.1
16:1	Palmitoleic acid	-
17:0	Margaric acid	0.1±1.3
18:0	Stearic acid	2.2±0.0
18:1	Oleic acid	0.2±2.7
18:2	Linoleic acid	0.1±1.3
18:3	Gamma-linolenic acid	0.1±1.3
18:3	Alpha-linolenic acid	0.2±2.7
20:0	Arachidic acid	0.1±1.3
20:1	Gadoleic acid	-
20:2	Eicosatrienoic acid	0.4±5.5
20:3	Arachidonic acid	0.5±0.0
20:4	Eicosapentaenoic acid	0.8±1.11
20:5	Behenic acid	0.1±1.3
22:5	Docosapentaenoic acid	7.3±0.0
22:6	Docosahexaenoic acid	30.1±3.5
24:0	Lignoceric acid	0.4±5.5

Number of replicates for experiment, n=3, Data given in mean±SD

The distillery wastewater, when used as a medium component, not only supported the growth of the medium but also facilitated the lipid production, as 2.9 g/l lipid was obtained at the end of 72 h. As the incubation time was further progressed, the *Schizochytrium* sp. was able to accumulate a maximum lipid (5.8 g/l) at the end of 144 h, which was initially 4.4 and 5.2 g/l at 96 and 120 h respectively.

After the fermentation a total nitrogen and COD of the culture sample was found to be 0.2 % and 7600 mg/l respectively, which showed the effective utilization of the nitrogen by the *Schizochytrium* sp.

Optimization of distillery wastewater concentration in batch cultivation

The concentration of distillery wastewater (DWW) required for the production of polyunsaturated fatty acids was an important parameter in studies related to usage of it as a medium component. The experiments when performed in batch cultivations using distillery wastewater as a component of media, the biomass and lipid production was similar as obtained in batch cultivation studies when peptone and yeast extract was used. However, the lipid yield (4.5 g/l) was observed when 100 % distillery wastewater was used. At lower concentrations (25 %, 50 % and 75 %) of distillery wastewater, the biomass obtained was 23.7, 31.9 and 43 g/l respectively. The yields of the biomass, lipid, and DHA produced by the Schizochytrium sp. have been presented in fig. 2.

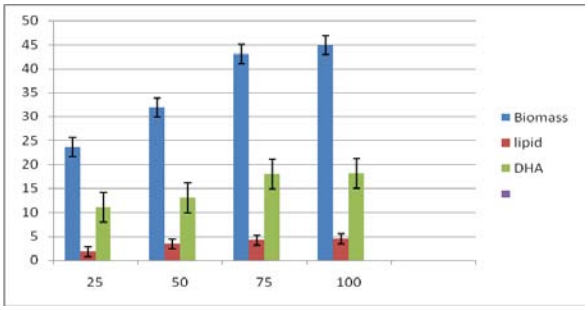

Fig. 2: Production of biomass, lipid (g/l) and DHA (% lipid w/w) at different concentrations of distillery wastewater in batch cultivations
Data are given in mean±SD

Carbon source optimization

The ability of *Schizochytrium* sp. to utilize various carbon sources when used in combination with distillery wastewater was similar to that observed in earlier experiments when yeast extract and peptone were used as nitrogen sources. The glucose was found as the most suitable carbon source in distillery wastewater medium. 56.7 and 5.8 g/l biomass and lipid yield respectively were obtained when glucose was used as the carbon source. It was observed that polysaccharides were not utilized as potential carbon sources as a low biomass (22.3 g/l) and lipid (1.7 g/l) was obtained with starch. The disaccharides supported the growth of the *Schizochytrium* sp. where a biomass of 44.9 g/l was obtained with maltose.

Optimization of carbon source concentration

Media containing distillery wastewater with various concentrations of glucose added in batches were evaluated in the present studies. The strain showed a good growth at a final glucose concentration (between 40 and 100 g/l), while a poor growth was observed in the absence of glucose (table 3). Maximum biomass (55.8 g/l) and lipid (5.4 g/l) were obtained when fed-batch was done with 1 % glucose. The biomass at 1.5 % fed-batch was similar to that obtained with 1 % glucose fed batch. However, the lipid production was low. The fed-batch with 2 % culture growth could gave a yield of biomass (36.5 g/l). A maximum yield of DHA was obtained with 1 % fed-batch (28.2 % DHA in lipid).

Effect of Sea salt concentration

In this study, the requirement of minimum concentration of sea salt used with distillery wastewater for proper growth and lipid production was investigated. It was found that the organism being marine origin was not able to survive in zero salinity. The results obtained at 17.5 g/l sea salt were similar with that obtained at 35 g/l sea salt concentration. A maximum biomass (57.3 g/l) and lipid (5.5 g/l) was observed when sea salt (17.5 g/l) was used in M6 medium. At lower salt concentration (4 and 8 g/l) although the growth of the organism could be achieved but the lipid yield was quite low (2.5 and 3.2 g/l) respectively (fig. 3).

Table 3: Effect of different concentrations of glucose fed batch in distillery wastewater medium on the production of biomass and lipid

Glucose (%)	Biomass (g/l)	Lipid (g/l)	Cell count (cells/ml/mm^3)	Residual glucose (g/l)
No carbon	18.2±0.0	0.9±0.0	5.9 x 10^6±8.8	Nil
0.5 %	47.1±0.0	3.7±4.4	7.7 x 10^7±0.0	1.8±0.0
1 %	55.8±7.1	5.4±8.8	9.8 x 10^7±0.0	3.5±0.0
1.5 %	52±0.0	4.5±0.0	8.6 x 10^7±0.0	7.2±0.0
2 %	36.5±0.0	2.8±4.4	6.2 x 10^7±0.0	18.5±0.0

Number of replicates of experiment, n=3, Data given in mean±SD

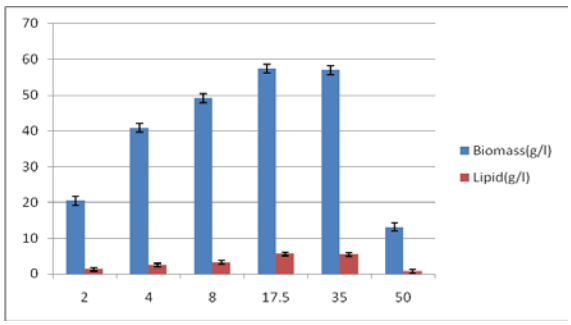

Fig. 3: Production of biomass and lipid at different concentration of sea salt in distillery wastewater medium
Data are given in mean±SD

Effect of pH

The effect of the initial pH of the culture medium on the production of biomass and lipids was a serious concern as low pH of the distillery wastewater might affect the microbial growth. Although the pH of the medium was adjusted, however, a decrease in the pH during the period of experimentation was observed (table 4). It was found that maximum biomass (56.9 g/l) and lipid (5.4 g/l) yields were obtained when pH of the medium was kept in the range of 7-8. At low pH the growth of the *Schizochytrium* sp. was found to be inhibited, as a low biomass (15.3 g/l) was obtained at pH 4. The basic pH 9 did not affect the biomass production but could do so for the production of lipid as it was found quite low (2.6 g/l).

Effect of incubation temperature, aeration, and agitation

Incubation temperature variation with respect to medium containing distillery wastewater followed the same pattern as observed in previous studies. The optical densities of the cultures, after 60 h of fermentation at 20 and 28 °C were found 4.2 and 5.0, respectively. Apparently, the growth was more stimulated at the higher temperature. The cultures when grown at 28 °C the lipid yield was 5.7 g/l as compared to 4.1 g/l obtained at 20 °C. The DHA content also varied with the incubation temperature as 28.2 % DHA in lipid was obtained at 28 °C (fig. 4).

Table 4: Effect of initial medium pH on cultivation of *Schizochytrium* sp. in distillery wastewater medium

Initial pH	Final pH	Wet Biomass (g/l)	Lipid (g/l)	Cell count (cells/ml/mm^3)
4	3.1	15.3±1.7	0.7±1.1	4.7 x 10^5±0.0
5	4.5	32.6±0.0	2.7±4.4	5.9 x 10^7±8.8
6	5.5	43.2±7.1	4.1±0.0	9.0 x 10^7±0.0
7	6.4	55.7±7.1	5.4±8.8	1.1 x 10^8±0.0
8	7.5	56.9±0.0	5.4±8.8	1.3 x 10^8±0.0
9	8.4	40.8±0.0	2.6±0.0	7.0 x 10^7±0.0

Number of replicates of each experiment,n=3, Data given in mean±SD

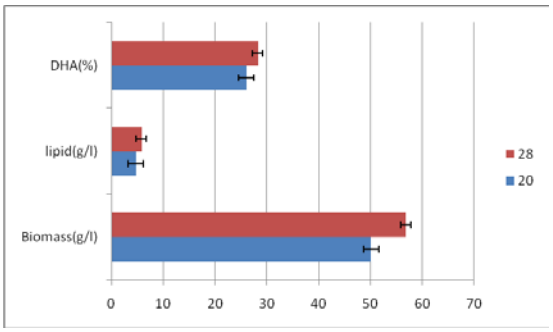

Fig. 4: Effect of temperature on biomass, lipid (g/l) and DHA (% in lipid w/w) production by *Schizochytrium* sp. in distillery wastewater medium
Data are given in mean±SD

In aerobic cultivations, the availability of oxygen is an important parameter for the growth of the culture. The effect of oxygen transfer rate on the production of biomass and lipid by *Schizochytrium* sp. was evaluated and presented in table 6. It was observed that when medium contained distillery wastewater as a major component, an OTR value (16 m mol/l/h) which corresponds to 200 ml volume in 1 L Erlenmeyer flasks produced maximum yield. The lipid production by *Schizochytrium* sp. exhibited a decline when the OTR value was reduced to 11 m mol/l/h. A higher OTR value (100 ml/1 L Erlenmeyer flask) resulted in the production of biomass (45 g/l), and lipid (3.7 g/l).

An intense aeration and mixing were required for the maximum production of biomass and lipid by *Schizochytrium* sp. Maximum yields of biomass (55.3 g/l) and lipid (5.4 g/l) were obtained when fermentation was carried out at 220 rpm. At lower rpm the growth of the organism was reduced resulting in a biomass and lipid yield of 29.3 and 2.5 g/l respectively (table 5).

Table 5: Effect of oxygen transfer rate (OTR) on the production of biomass and lipid by *Schizochytrium* sp. in distillery wastewater medium

Culture volume (ml)	Wet biomass (g/l)	Residual sugar (g/l)	Cell count (cells/ml/mm^3)	Total lipid (g/l)
100	39.0±0.0	5.33±0.0	7.5 x10^7±0.0	3.7±4.4
200	57.2±7.1	4.6±0.0	1.10 x10^8±0.0	5.4±8.8
300	46.9±0.0	10.7±1.7	9.3 x10^7±0.0	4.2±0.0

Number of replicates of each experiment, n=3, Data given in mean±SD

DISCUSSION

Alcohol distilleries are a major agro-based industry in India with around 300 units located mainly in rural and sugarcane growing regions. The total installed capacity is 3250 million L/alcohol/PA with an estimated production of 2300.4 million L in 2006–2007 (Ethanol India, 2007). Most of the distilleries co-exist with sugar mills which utilize the molasses from cane sugar manufacture as the starting material for alcohol production. The industry generates large volumes (8–15 kL/kL alcohol) (CPCB, 2002) of dark brown colored wastewater ("spent wash") with high BOD (45,000–60,000 mg/l) and COD (80,000–120,000 mg/l) [23].

Alcohol beverage distillery wastewater contains significant amounts of proteins and formal-type (free) amino acids (table 1). These nutrients can be utilized by the industrial microorganisms for the production of many valuable products. In this way, the pollution problem caused due to wastewater and cost reduction for the production of important products by a microorganism, both can be achieved. This study describes that the alcoholic beverage distillery wastewater contains ingredients which can be utilized by a marine *Schizochytrium* sp. *Schizochytrium* sp. has been reported to produce commercially important lipids docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA) the only medium components necessary for the growth of the strain were glucose and salts in addition to the distillery wastewater. Therefore, it can be said that the distillery wastewater can be used as a replacement for the conventionally used nitrogen sources such as peptone, yeast extract, and corn steep liquor at no extra cost, as the wastewater is almost freely available.

The concentration of distillery wastewater was an important parameter as more the percentage of distillery wastewater the strain can use more it will be economically feasible. The distillery wastewater (75 and 100 %) when used for the preparation of the medium produced maximum yields of biomass and lipid, with same DHA content. At this concentration of distillery wastewater the working percentage of total nitrogen (~3.0–4.0 g/l) was much higher than that (~0.2 g/l) in medium containing 1 % peptone and yeast extract. The results obtained in the present investigation were similar to that reported [22], in which 30-40 % DHA was obtained at 50 % and more percentage of distillery wastewater. In batch cultivations, the yield of biomass and lipid was similar to the results obtained in previous batch experiments.

The distillery wastewater contains many water soluble carbohydrates (table 1). The lipid production by *Schizochytrium* sp. in the medium containing no glucose was although low but when compared to poor biomass infers a positive effect. It is desirable to achieve the optimization of cultural conditions and breeding of the strain so that no glucose be required for the production of DHA. The biomass and lipid produced in distillery wastewater medium at a fed-batch of 1 % glucose were found higher when compared to the same conditions used with peptone and yeast extract as nitrogen sources.

The effect of sea salt concentrations revealed interesting results as the *Schizochytrium* sp was able to utilize a lesser concentration of sea salt (17.5 g/l) for maximum production of biomass and lipid. Although the growth of the organism in fed-batch cultivations using peptone and yeast extract was high, however, the lipid content of the biomass was low when compared to the experiment done with distillery wastewater. It can be inferred that the distillery wastewater contains many inorganic solids which facilitated the growth and accumulation of lipid by the strain in the study.

The pH of the initial medium affected the production of lipid and growth of the organism. Due to the low pH of the distillery wastewater, the base used for pH adjustment may have some interference with the biomass production. The optimum pH was found in the range of 7-8. Our results with distillery wastewater as a medium component produced maximum yield at pH 8. An optimum pH (5.5 to 7.0) has been reported for thraustochytrids [25, 26]. In our studies, the initial pH of the medium when kept at 6 lowered the production of biomass and lipid.

The effect of temperature and aeration was similar to that observed in previous experiments where 28 °C incubation temperatures was found to be optimum. The strain used in this study produced maximum biomass and lipid when an OTR value (16 m mol/l/h) was used. The requirements of high oxygen have been reported for thraustochytrids, particularly in *Schizochytrium* sp. [26].

CONCLUSION

The objective of this study was to decrease the disposal amounts of the alcoholic beverage distillery waste-derived carbon and nitrogen compounds, which would be beneficial for the preservation of the environment and further use them as a cheaper raw material for the production of docosa hexanoic acid. The DHA production in shake flasks after 120 h of incubation is 30.1 % with lipid accumulation of 53.6 g/l. It can be concluded that by optimization of media, pH, aeration and agitation in reactors it is possible to increase the yield of DHA. As it is generally possible to achieve an adequate aeration and mixing in bioreactor culture, the higher DHA production yields could be the result of an appropriate combination of all the above-given factors. The study indicates that *Schizochytrium sp* may be a potential organism for further development and optimization of a fermentation process as an alternative source for the production of DHA. The fermentation conditions can be optimized to increase the accumulation of desired unsaturated fatty acid and thus lowering the COD of industrial waste along with a proper selection of the microorganism can be achieved.

CONFLICT OF INTERESTS

Declare none

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