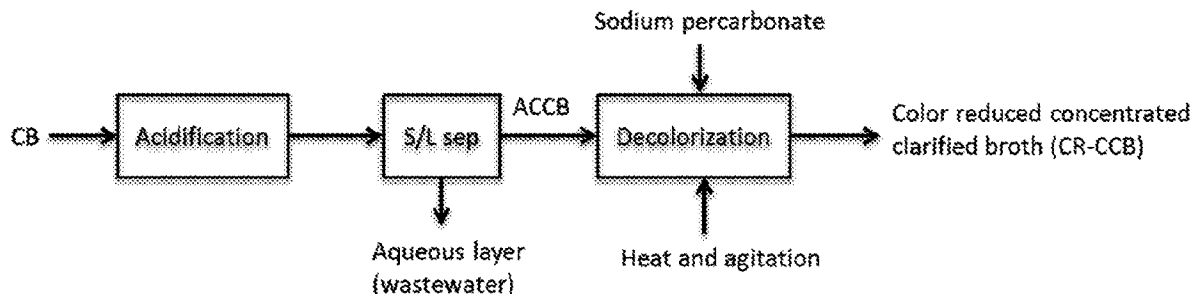




(86) Date de dépôt PCT/PCT Filing Date: 2017/06/01
 (87) Date publication PCT/PCT Publication Date: 2018/08/09
 (45) Date de délivrance/Issue Date: 2024/02/20
 (85) Entrée phase nationale/National Entry: 2019/08/01
 (86) N° demande PCT/PCT Application No.: US 2017/035403
 (87) N° publication PCT/PCT Publication No.: 2018/144053
 (30) Priorité/Priority: 2017/02/06 (US62/455,562)

(51) Cl.Int./Int.Cl. *C07H 15/06* (2006.01),
C11D 1/66 (2006.01)
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(54) Titre : DECOLORATION DE COMPOSITION CONCENTREE DE RHAMNOLIPIDES
 (54) Title: DECOLORIZATION OF CONCENTRATED RHAMNOLIPID COMPOSITION



(57) Abrégé/Abstract:

Provided is a method for decolorizing and optionally neutralizing a rhamnolipid composition comprising treating said composition with a percarbonate salt as well as the decolorized and optionally neutralized composition obtainable therefrom. Also provided ' a method for improving the quality of wastewater generated during said method.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau

(43) International Publication Date
09 August 2018 (09.08.2018)



(10) International Publication Number
WO 2018/144053 A1

(51) International Patent Classification:

C07H 15/06 (2006.01) C11D 1/66 (2006.01)

(21) International Application Number:

PCT/US2017/035403

(22) International Filing Date:

01 June 2017 (01.06.2017)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/455,562 06 February 2017 (06.02.2017) US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK,

EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

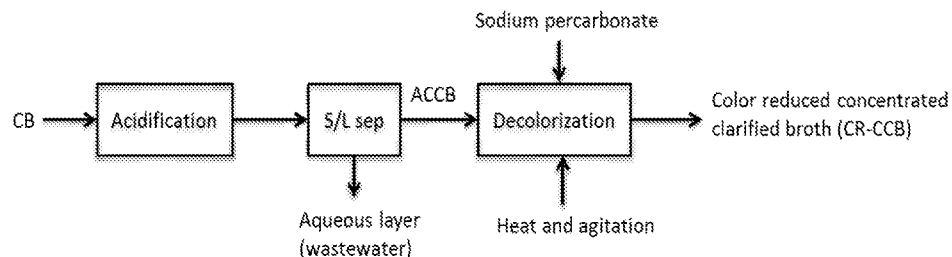
- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))

Published:

- with international search report (Art. 21(3))

(54) Title: DECOLORIZATION OF CONCENTRATED RHAMNOLIPID COMPOSITION

Figure 1



(57) Abstract: Provided is a method for decolorizing and optionally neutralizing a rhamnolipid composition comprising treating said composition with a percarbonate salt as well as the decolorized and optionally neutralized composition obtainable therefrom. Also provided ' a method for improving the quality of wastewater generated during said method.



WO 2018/144053 A1

DECOLORIZATION OF CONCENTRATED RHAMNOLIPID COMPOSITION

FIELD

5 Provided is a method for decolorizing and optionally neutralizing a rhamnolipid composition comprising treating said composition with a percarbonate salt as well as the decolorized and optionally neutralized composition obtainable therefrom. Also provided is a method for improving the quality of the liquid phase generated (also referred to as the aqueous layer or wastewater) which contains a variety of organic and/or inorganic substances during fermentation during said decolorization process.

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BACKGROUND

The surface-acting agents or surfactants play an essential role in an industrial application especially personal cares and household cleaning products due to their ability to foam, clean, disperse, emulsify and lower surface tensions. Most surfactants available in the market are chemically derived from petroleum. Therefore, the demand of biosurfactants has been significantly increased due to their eco-friendly properties (i.e., biodegradability and biocompatibility) [1].

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Rhamnolipids (RLs) are one of the most investigated and well known biosurfactants since they have high biodegradability, low toxicity, high foaming and the ability to be synthesized from renewable feedstock [2-4]. Rhamnolipids are interface-active glycolipids containing carbohydrates (rhamnose) and aliphatic acids (hydroxy fatty acids). RLs consist of one (monorhamnosylipids or mono-rhamnolipids) or two rhamnose units (dirhamnosylipids or di-rhamnolipids) and one or two (predominantly two) 3-hydroxy fatty acid residues. Several industrial applications include detergents and cleaners, antifungal [5, 6], cosmetics, bioremediation and enhanced oil recovery (EOR) [7].

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The production of rhamnolipids includes an aerobic fermentation of bacteria predominately *Pseudomonas aeruginosa* with various carbon and nitrogen sources such as vegetable oil, crude glycerol, molasses, soap stock [8, 9]. The RL concentration is typically in the range of 1-50 g/L depending upon types of feedstock, fermentation condition and fermentation process [10-12]. Since the RL concentration obtained from the fermentation is very low, separation and purification are required afterwards to produce concentrated RL product (i.e., 30-90%RL concentration). The most common separation process starts with an acid precipitation. By adjusting the fermentation broth pH to 2-3 making the

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rhamnolipids insoluble in the aqueous solution and thus, precipitate out. A solvent extraction with ethyl acetate, hexane, chloroform-methanol, butanol [13, 14] is also performed to further purify rhamnolipids from aqueous layer which is discarded. After stripping the solvent out [15], a concentrated viscous brownish-to-black oily rhamnolipid liquid is obtained.

However, only a small quantity of rhamnolipids is available on the market at high price. It is very hard to find the availability online without submitting a request order form. Among those listed for sale, the concentration of rhamnolipids with 90% or less contains dark brown color [16, 17]. The brownish-to-black color of concentrated rhamnolipids is possible due to the pigment, pyocyanin, produced by *P. aeruginosa* during fermentation [18-20]. The dark color of the concentrated rhamnolipids appears to be an issue when being used as a biosurfactant for personal care and household cleaning products. The current possible route to remove this color is to purify the concentrated rhamnolipids further using a thin layer chromatography (TLC) to remove color but the process is costly and time consuming which is unnecessary to do so for the personal cares and household cleaning application since the concentration of rhamnolipids used in these products is very low (less than 5%).

After separating rhamnolipids out, the aqueous layer is sent to a wastewater treatment plant since it contains a variety of inorganic and organic substances unconsumed during fermentation. The most commonly measured quality of wastewater is the biochemical oxygen demand (BOD). The higher the BOD, the higher organic matters in water thus, required more oxygen to break down those compounds which means a higher operating cost for the water treatment. The measurement for the total suspended solid (TSS) is also used to qualify the quality of the wastewater since they cause problem to aquatic life.

SUMMARY

Provided is an organic solvent-free process for obtaining a neutralized composition comprising one or more rhamnolipids (RLs) comprising: (a) providing an aqueous medium comprising at least one rhamnolipid; (b) treating said medium provided with acid to obtain an acidic medium comprising a solid, liquid and oily phase; (c) removing at least the liquid phase from said acidic medium of (b) to obtain an acidic solid phase and optionally acidic oily phase and (d) treating said acidic solid phase and optionally acidic oily phase obtained

in (c) with a composition comprising a percarbonate salt in an amount effective to neutralize and decolorize said acidic solid phase and optionally said acidic oil phase of step (c) to obtain said neutralized composition. Also provided is a composition comprising a neutralized and decolorized solution obtainable therefrom. In a particular embodiment, the neutralization and decolorization step occurs at a temperature of between about 30-80C for at least about 30 minutes and in a more particular embodiment, to about 1 week. In a particular embodiment, the composition used in step (d) to decolorize and neutralize may further comprises a second base, e.g., NaOH. In a particular embodiment, percarbonate salt is present in a concentration of about 0.5-3% w/w equivalent hydrogen peroxide concentration (EHPC).

Also provided is a method for increasing the quality of the liquid phase obtained in step (b) comprising treating said liquid phase with an amount of percarbonate salt effective to increase said quality of the liquid phase. The quality of the liquid phase may be determined by measuring the biochemical oxygen demand (BOD) and/or total suspended solid (TSS). In a particular embodiment, provided is a method of reducing the BOD and/or TSS of at least about 25% in the liquid phase comprising treating said liquid phase with a composition comprising a percarbonate salt in an amount effective to reduce BOD and/or TSS of at least about 25% in said liquid phase. In a more particular embodiment, BOD and/or TSS is reduced at least about 30%, more particularly at least about 40%, even more particularly at least about 45%, yet even more particularly about 50%, yet even more particularly at least about 55% and even yet more particularly at least about 60%, wherein percarbonate salt is present at a concentration of about 0.2% to about 0.8% w/w EHPC.

DEFINITIONS

Where a range of values are provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either both of those included limits are also included in the invention.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, the preferred methods and materials are now described.

Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention. To the extent the material cited herein contradicts or is inconsistent with this specification, the specification will supersede any such material.

It must be noted that as used herein, the singular forms "a," "and" and "the" include plural references unless the context clearly dictates otherwise.

Unless otherwise indicated, the term "at least" preceding a series of elements is to be understood to refer to every element in the series. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the present invention. Throughout this specification which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integer or step. Thus the terms "comprising", "including", "containing", "having" etc. shall be read expansively or open-ended and without limitation. When used herein, the term "comprising" can be substituted with the term "containing" or sometimes when used herein with the term "having".

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 schematically shows an overall process producing color reduced concentrated clarified broth (CR-CCB) with sodium percarbonate treated acid-form concentrated clarified broth (ACCB).

Figure 2 schematically shows the reaction scheme used in Example 2 of US appln. ser no. 14992995, filed Jan. 11, 2016 (published as US20160272667).

Figure 3 shows a comparison of the color of the samples with sodium percarbonate addition at 0.75, 1, 1.25, 1.5, 2, 2.5 and 3% EHPC (from left to right). Pictures were taken at 30 min of heating at 60C and 250 rpm.

Figure 4 shows a comparison of the color of the samples with sodium percarbonate addition at 0.75, 1, 1.25, 1.5, 2, 2.5 and 3%EHPC (from left to right). Pictures were taken at 3 hours of heating at 60C, 250 rpm.

Figure 5 shows the color of CCB obtained from Example 2 of US20160272667 (0% EHPC, 1st left) vs. the color of CR-CCB obtained from sodium percarbonate addition at 2, 2.5 and 3%EHPC after heating at 60C, 250 rpm for 30 min (from 2nd left to right).

Figure 6 shows the optical density of CCB sample (0%EHPC) and CR-CCB with various sodium percarbonate concentrations (2-3% EHPC) after heating at 60C for 3 hours.

Figure 7 shows the optical density of CCB sample (0%EHPC) and CR-CCB with various sodium percarbonate concentrations (1-2.5% EHPC) after heating at 45C for 4 hours.

Figure 8 shows the %reduction in optical density (color) of CR-CCB samples with 1-2.5% EHPC compared to no sodium percarbonate addition (0%EHPC) after heating at 45C for 4 hours.

DESCRIPTION OF SPECIFIC EMBODIMENTS

Provided is a process to reduce color of concentrated rhamnolipid solution (30-90%) using a sodium percarbonate ($\text{Na}_2\text{CO}_3 \cdot 1.5 \text{H}_2\text{O}_2$). The starting material is an acidic rhamnolipid slurry (the solid rhamnolipid containing phases) obtained from the precipitation of rhamnolipid out of clarified broth at about pH 2.1 as described in US appln ser no. 14992995, filed Jan. 11, 2016 (published as US20160272667). The sodium percarbonate is added into the acidic rhamnolipid slurry at about 2-3% w/w equivalent hydrogen peroxide concentration (EHPC). Additional base such as NaOH can be used at about 0.5-1.5% w/w to bring the pH neutral if needed. Heating the solution at about 40-60 C for 30 min to several hours can speed up the decolorization process. After heating, the color of the concentrated rhamnolipids is lightened from black color like molasses to light brown or dark yellow color like honey. There is no loss of rhamnolipid concentration as a result of heating the solution.

Further provided as set forth in Examples 2 and 3 is a process to increasing (alternatively referred to as enhancing) the quality of the liquid phase obtained. The

increase in the quality of the liquid phase may be determined by BOD and/or TSS and/or OD measurements between about OD400 to about OD800.

Although US20160272667 discloses the decolorization of the rhamnolipids obtained from an organic solvent-free process with peroxide treatment using hydrogen peroxide (H₂O₂) or organic peroxide (peroxyacetic acid) or inorganic peroxide (sodium peroxide), the peroxides proposed disclosed may have a high risk of flammability, health and reactivity based on the degree of hazard listed on NAFA 704 [21]. Unlike the abovementioned chemicals, sodium percarbonate is an eco-friendly chemical that has been used in the household word wide commercially named as “Oxyclean”. The advantages of using a sodium percarbonate as a decoloring agent over the peroxides are:

1. Safer to transport since the sodium percarbonate is a soda ash with hydrogen peroxide combined in the stable white powder form;
2. The decolorization with sodium percarbonate takes place simultaneously with the neutralization step and thus, the process is shorter as compared to the decolorization step described in US20160272667;
3. The working volume is significantly reduced compared to US20160272667 since the starting material is the concentrated rhamnolipid solution not the aqueous medium (e.g., fermentation medium or broth) and thus, only a small volume being treated;
4. The process is significantly shorter. The process is done within 1 hour compared to 48 hours.

The process described here can be used in any fermentation product that is dark in color due to pigments produced by microorganisms.

EXAMPLES**Example 1: Color removal of acid-form concentrated clarified broth (ACCB) with sodium percarbonate addition at 0.75-3% w/w equivalent hydrogen peroxide concentration (EHPC) at 60C**

The starting material of this process is an acid-form concentrated clarified broth (ACCB) obtained from the precipitation of rhamnolipid out of clarified broth (CB) at pH 2.1 before neutralization step as described in US20160272667.

The pH of the starting material, ACCB, is around 2.1. Sodium percarbonate having a chemical formula of $\text{Na}_2\text{CO}_3 \cdot 1.5\text{H}_2\text{O}_2$ is added to ACCB at 0.75-3% w/w equivalent hydrogen peroxide concentration (EHPC). The samples are heated at 60C with agitation at 250 rpm using MaxQ™ 8000 Stackable Orbital Shakers (Thermo Scientific) to ensure consistency of the agitation speed and temperatures of all samples.

A reduction in color of the samples with sodium percarbonate addition are shown in Figures 3 and 4.

Sodium hydroxide (NaOH) is added to the samples with sodium percarbonate addition less than 2%EHPC in order to bring the pH ~7. The amount of sodium percarbonate and NaOH added to the samples are shown in Table 1 along with the concentration of residue H_2O_2 in the samples after 4 hours of heating at 60C.

Table 1: The amount of sodium percarbonate, NaOH and hydrogen peroxide in the samples

%EHPC	%Na percarbonate	%NaOH	pH	%H₂O₂ residue	%H₂O₂ consumption
0.75%	2.3%	3.6%	6.9	0.50%	33%
1.00%	3.1%	1.9%	6.9	0.55%	45%
1.25%	3.8%	1.7%	7.0	0.65%	48%
1.50%	4.6%	1.5%	7.1	0.69%	54%
1.75%	5.4%	1.2%	7.4	0.78%	55%
2.00%	6.2%	1.0%	7.6	0.63%	69%
2.50%	7.7%	0%	7.7	0.25%	90%
3.00%	9.2%	0%	8.1	0.18%	94%

The color of the concentrated clarified broth (CCB) is significantly reduced with the addition of sodium percarbonate from dark brown to very light yellow as seen in the Figure 5.

5 The quantity of light absorbance or optical density (OD) of samples is measured at various wavelengths using a GENESYS™ 20 Visible Spectrophotometers (Thermo Scientific) in order to compare the concentration of light-absorbing constituent in the medium samples. Based on Beer's and Lambert's Laws, the higher the OD, the darker the sample. Figure 6 clearly shows that the CCB sample designated as 0%EHPC (the 1st left sample in Figure 5) has the highest OD than the other samples with sodium percarbonate addition. By diluting the CCB sample (0%EHPC) 5 times with deionized water, the OD 10 appears to be in the same range as those reduced color concentrated clarified broth (CR-CCB). This suggests that the color of the CR-CCB is relatively about the same darkest as 20% CCB. Please note that the starting material ACCB for this example is taken from the same batch/source and thus, the variation of the material is zero.

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Example 2: Color removal of acid-form concentrated clarified broth (ACCB) with sodium percarbonate addition at 1%-2.5% w/w equivalent hydrogen peroxide concentration (EHPC) at 45C

20 The starting material of this process is an acid-form concentrated clarified broth (ACCB) as described in Example 1. Sodium percarbonate is added to the starting material, ACCB, at 1%, 1.5%, 2% and 2.5% w/w equivalent hydrogen peroxide concentration (EHPC). Sodium hydroxide (NaOH) is also added to the samples with sodium percarbonate addition less than 2.5%EHPC in order to bring the pH ~7.

25 The samples are then heated at 45C in non-baffle flasks with agitation at 350 rpm using MaxQ™ 8000 Stackable Orbital Shakers (Thermo Scientific) to ensure consistency of the agitation speed and temperatures of all samples. The degree of darkness of the samples is quantified with a GENESYS™ 20 Visible Spectrophotometers (Thermo Scientific) to measure the optical density (OD) of the samples at various wavelengths. The lower the OD value, the lighter the sample color. The optical density of the samples is shown in Figure 7. 30 The darkness color of the sample reduced with increasing the concentration of sodium percarbonate addition so as the OD value. The % reduction in optical density or color of the samples is also calculated against the sample without sodium percarbonate (0%EHPC) and plotted in Figure 8.

Example 3: Color removal and wastewater quality enhancement of the aqueous layer waste stream from non-organic solvent rhamnolipid concentrated process by acid precipitation described in US20160272667 with sodium percarbonate addition

5 The aqueous (water) layer obtained from acid precipitation step of clarified broth (CB) to obtain concentrated rhamnolipid products described in Examples 2-6 of US20160272667 is considered a wastewater from this process which is a starting material for this example. Sodium percarbonate is added into this acidic wastewater stream at 0.25% and 0.5% EHPC at 60C. After 1 hour of gentle agitation at 60C, the samples are removed
10 from heat and let to cool to room temperatures during 1-2 hours. Afterwards, the samples are subjected to optical density measurement and biological oxygen demand (BOD) and total suspended solid (TSS) analysis. BOD

The optical density (OD) of the samples is measured with a GENESYS™ 20 Visible Spectrophotometers (Thermo Scientific). BOD and TSS are performed based on Standard
15 Methods for the Examination of Water and Wastewater (available from www.standardmethods.org) 5210B and 2540, respectively). The % optical density at various wavelength (nm), %BOD and %TSS reductions of the samples are shown in Table 2. It clearly shows that treating the aqueous waste stream with sodium percarbonate at 0.5%EHPC gives rise to the highest reduction of BOD and TSS while the most reduction in
20 color is observed with 0.75%EHPC sample at the studied wavelengths (400-800 nm). The color of the aqueous layer is reduced with the addition of sodium percarbonate from brown to yellow.

Table 2: % Reduction of optical density at various wavelength, BOD and TSS of samples with sodium percarbonate addition. For example, OD 400 indicates optical density of samples at 400 nm.

%EHPC	%Reduction						
	OD 400	OD 500	OD 600	OD 700	OD 800	BOD	TSS
0.25%	0%	0%	38%	52%	63%	40%	45%
0.50%	32%	72%	88%	92%	99%	68%	53%
0.75%	78%	90%	95%	95%	96%	28%	-

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WHAT IS CLAIMED IS:

1. An organic solvent-free process for obtaining neutralized and decolorized rhamnolipids (RLs), the organic solvent-free process comprising:

(a) providing an aqueous medium comprising at least one rhamnolipid;

(b) treating said medium provided with acid to obtain an acidic medium comprising solid, liquid and oily phases;

(c) removing at least the liquid phase from said acidic medium of (b) to obtain an acidic solid phase and optionally an acidic oily phase;

(d) treating said acidic solid phase and optionally said acidic oily phase obtained in (c) with a percarbonate salt, wherein said percarbonate salt is in an amount effective to neutralize and decolorize said acidic solid phase and optionally said acidic oily phase to obtain said neutralized and decolorized rhamnolipids,

wherein said neutralization and decolorization occurs at a temperature of between 35-85°C for at least 30 minutes.

2. The organic solvent-free process according to claim 1, wherein said percarbonate salt in step (d) is present in an amount of 0.5% to 3% w/w equivalent hydrogen peroxide concentration (EHPC).

3. A method for increasing the quality of the liquid phase removed in step (c) in the process of claim 1, comprising treating said liquid phase with an amount of percarbonate salt effective to increase said quality.

4. The organic solvent-free process according to claim 1, further comprising treating the liquid phase removed in step (c) with a percarbonate salt in an amount effective to reduce biochemical oxygen demand (BOD) and/or total suspended solids (TSS) by at least 25% in said liquid phase.

5. The process according to claim 4, wherein said liquid phase is treated with 0.2% to 0.8% w/w EHPC percarbonate salt.

6. The process according to claim 1, wherein said percarbonate salt is combined with a second base to treat said acidic solid phase and optionally said acidic oily phase in step (d).

7. The process according to claim 1, wherein said neutralization and decolorization occurs at a temperature of between 45-60°C within 0.5 to 4 hours.

Figure 1

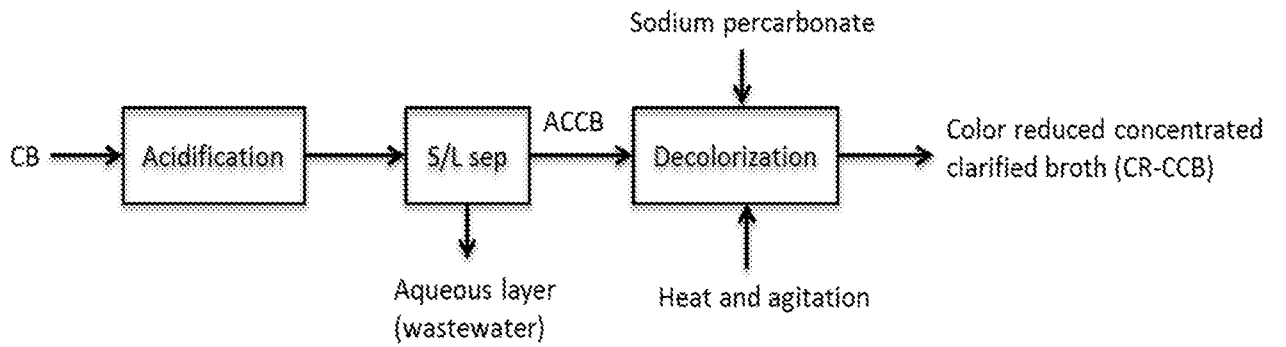


Figure 2

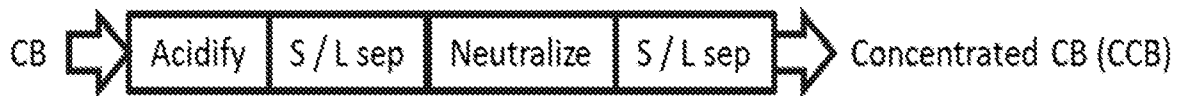


Figure 3

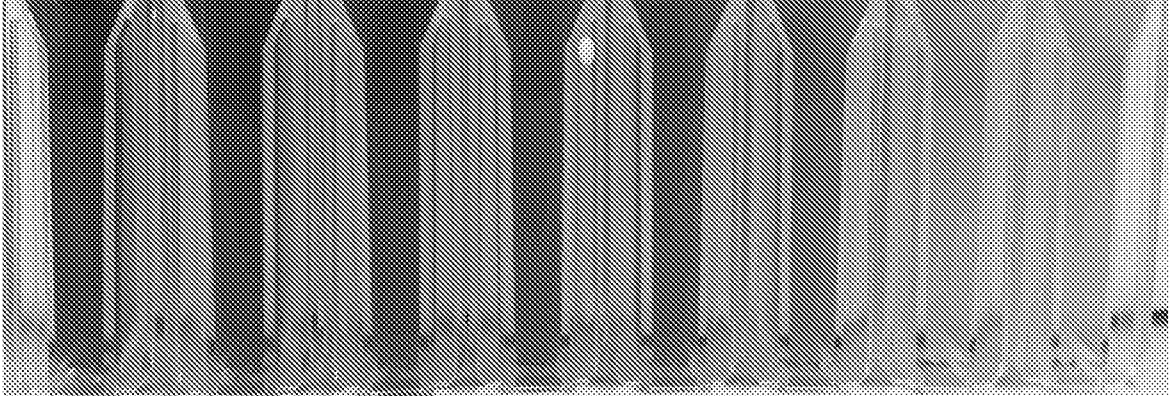


Fig. 4

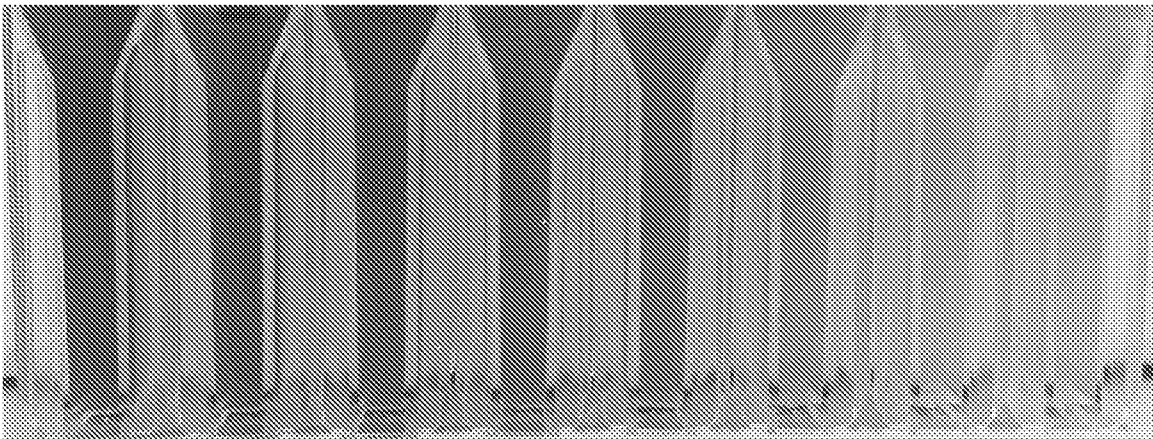


Figure 5

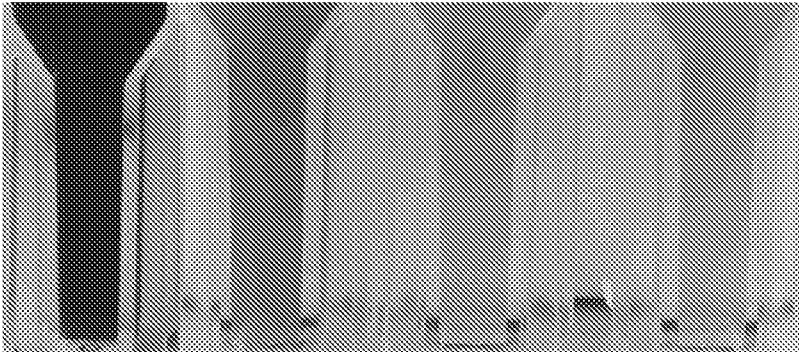
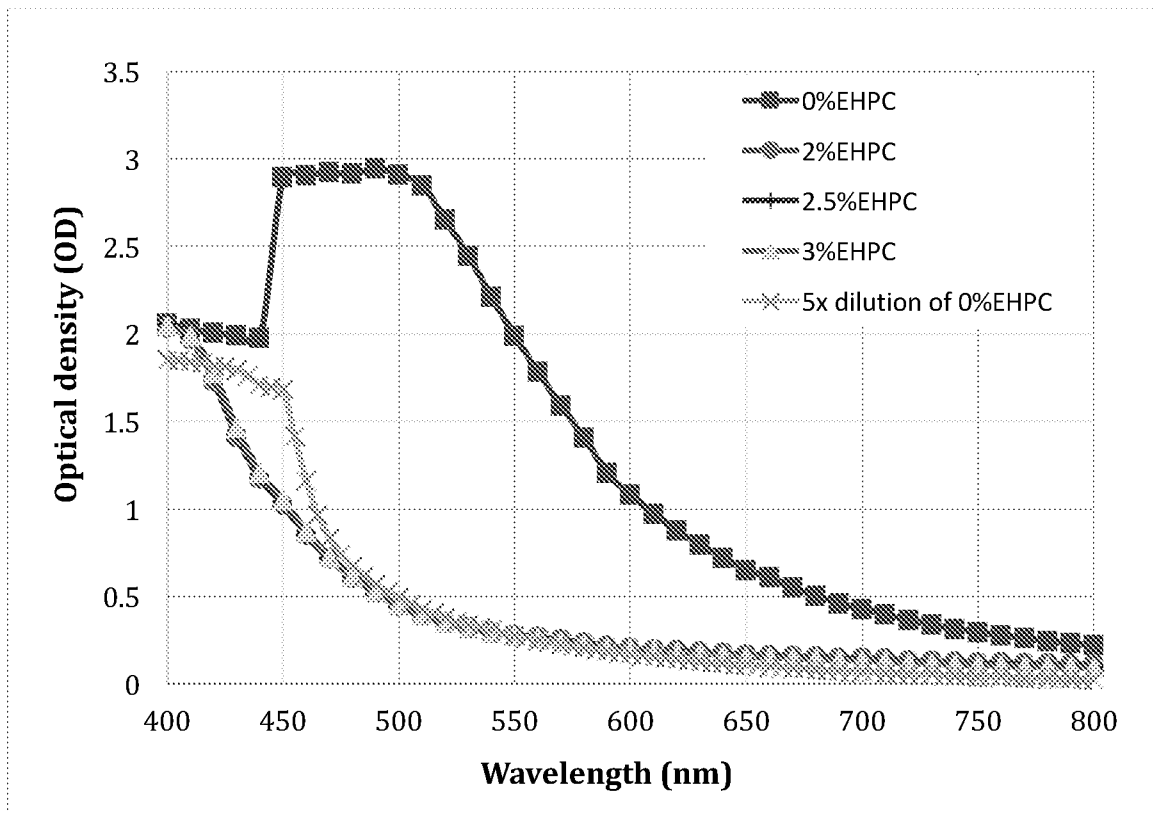


Figure 6



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Figure 7

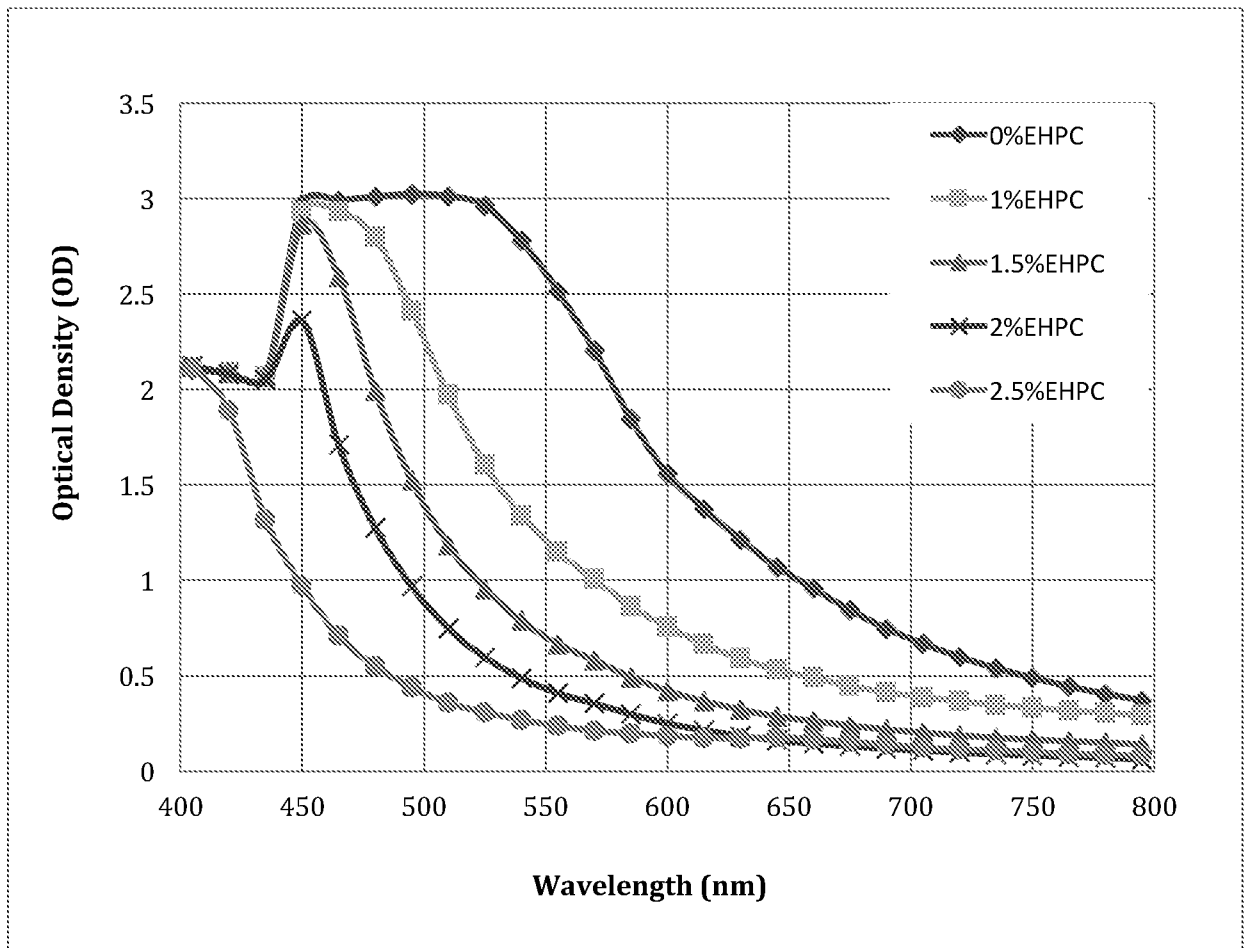


Figure 8